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Fukuoka, Japan. Kyushu Univ. Dept. of Fish
& the Fish. Res. Lab.
#2 Contributions.

Contributions from the Department of Fisheries

and

the Fishery Research Laboratory, Kyushu University

No. 2 (1955-1956)

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Foreword

After the Department of Fisheries and the attached Fishery Research Laboratory of Kyushu University were founded in 1941 and 1944, respectively, the research activities were greatly inhibited due to the outbreak of World War II and the restoration from its damage. After the War, activities were resumed and an increasing number of scientific papers have been published in various journals and bulletins.

It is, of course, difficult to obtain all of the available reports on the science of fisheries, particularly those which are carried out in Japan. This is perhaps one of the reasons why there are frequent duplication in and neglect of related works in this field.

It is hoped that this bulletin which is a compilation of all the scientific achievements from our institute will render our research works available to fisheries research organizations in foreign countries. The exchange of your scientific publications with this bulletin will be acknowledged with appreciation and will serve to promote the advancement of the science of fisheries in the whole world.

September, 1959

Department of Fisheries and
Fishery Research Laboratory,
Kyushu University,
Fukuoka, Japan

List of periodicals wherein published

Botanical Magazine, Tokyo	(植物学雑誌)
Breviora	
Bulletin of the Biogeographical Society of Japan	(日本生物地理学会々報)
Bulletin of the Japanese Society of Scientific Fisheries	(日本水産学会誌)
Collecting and Breeding	(採集と飼育)
Japanese Journal of Ecology	(日本生態学会誌)
Japanese Journal of Ichthyology	(魚類学雑誌)
Japanese Journal of Limnology	(陸水学雑誌)
Journal of the Agricultural Chemical Society of Japan	(日本農芸化学会誌)
Journal of the Faculty of Agriculture, Kyushu University	(九大農学部紀要)
Journal of Japanese Botany	(植物研究雑誌)
Journal of the Oceanographical Society of Japan	
Kagaku	(科学)
Nutrition and Food	
Proceedings of the Japan Academy	(日本学士院紀事)
Proceedings of the 7 th Pacific Science Congress	
Publications of the Seto Marine Biological Laboratory	
Records of Oceanographic Works in Japan	
Research in the effects and influences of the Nuclear Bomb Test Explosions (Tokyo)	
Science Bulletin of the Faculty of Agriculture, Kyushu University	(九大農学部学芸雑誌)
Seibutsu	(生物)

Contributions from the Department of Fisheries and the Fishery Research Laboratory, Kyushu University. No. 2 (1955～1956)

Paper with asterisk is not included in this series.

—— Biological Section ——

*Aikawa, H., Isobe, K.	On the first larvae of *Parribucus ursus-major* (Herbst)	Records Oceanogr. Works Japan, **2** (2) : 1～2 (1955)
*Dôtu, Y.	Life history of a goby, *Gobius poecilichthys* Jordan et Snyder クモハゼの生活史	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (1) 77～86 (1955)
*Dôtu, Y., Mito, S.	Life history of a gobioid fish, *Sicydium japonicum* Tanaka ボウズハゼの生活史	Ibid., **15** (2) : 213～221 (1955)
*Dôtu, Y.	The life history of a goby, *Chaenogobius urotaenia* (Hilgendorf) ウキゴリの生活史	Ibid., **15** (3) : 267～274 (1955)
*Dôtu, Y., Mito, S., Ueno, M.	The life history of a goby, *Chaeturichthys hexanema* Bleeker アカハゼの生活史	Ibid., **15** (3) : 359～365 (1955)
*Dôtu, Y.	On the life history of a gobioid fish, *Eutaeniichthys gilli* Jordan et Snyder ヒモハゼの生活史	Bull. Biogeogr. Soc. Japan, **16～19** : 338～344 (1955)
*Dôtu, Y., Mito, S.	On the breeding-habits, larvae and young of a goby, *Acanthogobius flavimanus* (Temminck et Schlegel) マハゼの産卵習性および仔，稚魚について	Jap. J. Ichthyol., **4** (4～6) : 153～161 (1955)
*Fujita, S., Nakahara, K.	On the development of the egg and prelarval stages of a scorpaenid fish, *Inimicus japonicus* (Cuvier et Valenciennes) オニオコゼの卵発生と仔魚前期	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (2) : 223～228 (1955)

*Fujita, S.	On the development and prelarval stages of the file-fish, *Monacanthus cirrhifer* Temminck et Schlegel カワハギの卵発生と仔魚前期	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (2) : 229～234 (1955)
*Mito, S.	Breeding habits of a percoid fish, *Plesiops semeion* タナバタウオの産卵習性	Ibid., **15** (1) : 95～99 (1955)
Sawada, T.	Some observations on *Sargassum filicinum* Harvey シダモクに関する二三観察	Ibid., **15** (1) : 71～76 (1955)
Segawa, S., Ogata, E., Sawada, T.	Studies on the carpospore liberation in *Gracilaria verrucosa* (Huds.) Papenfuss I. Carpospore liberation accompanied with the half-drying オゴノリ果胞子の放出に関する研究 第1報　蔭干に伴う果胞子放出について	Ibid., **15** (2) : 235～243 (1955)
Segawa, S., Ogata, E., Sawada, T.	Studies on the carpospore liberation in *Gracilaria verrucosa* (Huds.) Papenfuss II. On the mechanism of carpospore liberation オゴノリ果胞子の放出に関する研究 第2報　果胞子放出の機作について	Ibid., **15** (2) : 245～254 (1955)
*Ueno, M., Nakahara, K.	On the development of eggs and rearing of larvae of a flying fish, *Cypselurus starksi* アリアケトビウオの人工授精孵化と仔魚飼育	Ibid., **15** (1) : 87～94 (1955)
Segawa, S.	Systematic anatomy of the articulated Corallines (Supplementary report). The structure and reproduction of *Yamadaia melobesioides* Segawa 有節サンゴモの解剖分類学的研究（追報） サビモドキ（新属新種）の構造と生殖	Bot. Mag. Tokyo, **68** (807) : 241～247 (1955)
*Uchida, K., Tsukahara, H.	The fish-fauna of Ariake Sound 有明海の魚類相について	Bull. Biogeogr. Soc. Japan, **16**～**19** : 292～302 (1955)
*Dôtu, Y.	On the habits and egg-development of a goby, *Pterogobius zonoleucus* Jordan et Snyder チヤガラの生態及び人工授精による卵発生	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (4) : 483～487 (1956)

*Dôtu, Y.	The life history of an eleotrid goby, *Parioglossus taeniatus* Regan サツキハゼ（新称）の生活史	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (4) : 489～496 (1956)
*Fujita, S., Ueno, M.	On the egg development and prelarval stages of *Platycephalus indicus* (Linné) コチの卵発生と仔魚前期	Ibid., **15** (4) : 513～518 (1956)
*Fujita, S., Ueno, M.	On the egg development and prelarval stages of *Fugu* (*Torafugu*) *rubripes rubripes* (Temminck et Schlegel) トラフグの卵発生と仔魚前期	Ibid., **15** (4) : 519～524 (1956)
*Fujita, S.	On the development of the egg and prelarval stages of the puffer, *Fugu* (*Shosaifugu*) *stictonotus* (Temminck et Schlegel) ゴマフグの卵発生と仔魚前期	Ibid., **15** (4) : 525～530 (1569)
*Fujita, S.	On the development of the egg and prelarval stages of the puffer, *Fugu* (*Shosaifugu*) *poecilonotus* (Temminck et Schlegel) コモンフグの卵発生と仔魚前期	Ibid., **15** (4) : 531～536 (1956)
*Fujita, S.	On the development of the egg and prelarval stages of *Nibea argentata* (Houttuyn) シログチの卵発生と仔魚前期	Ibid., **15** (4) : 537～540 (1956)
*Mito, S.	On the egg development and hatched larvae of *Lethrinus nematacanthus* Bleeker イトフエフキの卵発生と仔魚期	Ibid., **15** (4) : 497～500 (1956)
*Mito, S.	On the development of the eggs and hatched larvae of *Oplegnathus fasciatus* (Temminck et Schlegel) イシダイの卵発生と仔魚期	Ibid., **15** (4) : 501～506 (1956)
*Mito S.	On the development of the eggs and hatched larvae of a Trachinoid fish, *Neoperis sexfasciata* (Temminck et Schlegel) クラカケギスの卵発生と仔魚期	Ibid., **15** (4) : 507～512 (1956)
Sawada, T.	Some observations on *Sargassum filicinum* Harvey (?) and its embryo-development シダモク（？）に関する観察及びその胚発生	Ibid., **15** (4) : 541～549 (1956)

TOMIYAMA, T., etc.	Studies on utililization of wastes inprocessing shell-fish I. Effect on chick growth and egg production 貝類廃棄部の利用に関する研究 Ⅰ. 鶏の生長及び産卵に及ぼす効果	Bull. Japan. Soc. Sci. Fisheries, **21** (6) : 429～432 (1955)
TOMIYAMA, T., YONE, Y., KATO, Y., OKAMOTO, M., YAMAOKA, M., KOYANAGI, T., UCHIDA, S., MURAKAMI, Y., TANAKA, S.	Studies on utilization of wastes inprocessing shell-fish II. The effect of the boiled extract of *Venerupis philippinarum* upon chick growth and increase of egg production during the molting season 貝類廃棄部の利用に関する研究 Ⅱ. 鶏の生長及び産卵率に及ぼすアサリ煮汁の効果	Ibid., **21** (6) : 433～436 (1955)
TOMIYAMA, T., etc.	Studies on utilization of wastes inprocessing shell-fish III. Fractionation of growth factors in extract of *Venerupis philippinarum* 貝類廃棄部の利用に関する研究 Ⅲ. アサリ煮汁の APF の分別	Ibid., **21** (8) : 937～940 (1955)
TOMIYAMA, T., etc.	Studies on utilization of wastes in processing shell-fish IV. The effect of viscera-extract of *Pecten laqueatus* on chick growth 貝類廃棄部の利用に関する研究 Ⅳ. 鶏の生長に及ぼすイタヤ貝内臓液化物の効果	Ibid., **21** (6) : 1181～1184 (1955)
TOMIYAMA, T., etc.	Studies on utilization of wastes in processing shell-fish V. The effect of viscera-extract of *Atrinapectinata japonica* on chick growth and egg production 貝類廃棄部の利用に関する研究 Ⅴ. 鶏の生長及び産卵に及ぼすタイラギ内臓液化物の効果	Ibid., **22** (6) : 374～377 (1956)
TOMIYAMA, T., YONE, Y., HIROWATARI, K., HARADA, Y., KOYANAGI, T., UCHIDA, S., TOKUDA, H.	Studies on utilization of trimmings wasted in manufacturing cooked fish paste - I. Effect of solubilized trimmings on chick-growth and egg production 煉製品製造残滓の利用に関する研究 Ⅰ. 鶏の生長及び産卵に及ぼすアラ液化物の効果	Ibid., **21** (8) : 941～944 (1955)

TOMIYAMA, T., YONE, Y., HIROWATARI, K., KOYANAGI, T., UCHIDA, S., TOKUDA, H.	Studies on utilization of trimmings wasted in manufacturing cooked fish paste - II. A long-term experiment on effect of solubilized trimmings on growth and egg production 煉製品製造残滓の利用に関する研究 Ⅱ. 鶏の生長及び産卵に及ぼすアラ液化物の長期投与の効果	Bull. Japan. Soc. Sci. Fisheries, **21** (10): 1086～1089 (1956)
TOMIYAMA, T., YONE, Y., HIROWATARI, K., KOYANAGI, T., UCHIDA, S., TOKUDA, H.	Studies on utilization of trimmings wasted in manufacturing cooked fish paste - III. Effectiveness of solubilized trimmings to prevention of lowering of the egg production in molting season 煉製品製造残滓の利用に関する研究 Ⅲ. 換羽期の産卵率低下の防止に対するアラ液化物の効果	Ibid., **21** (10): 1090～1092 (1956)
TOMIYAMA, T., YONE, Y., HIROWATARI, K., KOYANAGI, T., UCHIDA, S., TOKUDA, H.	Studies on utilization of trimmings wasted in manufacturing "Kamaboko", a cooked fish cake - IV. A large-scale experiment on the effectiveness of solubilized trimmings on egg production 煉製品製造残滓の利用に関する研究 Ⅳ. 残滓液化物による産卵上昇効果に関する実地試験	Ibid., **21** (10): 1093～1096 (1956)
TOMIYAMA, T., KAWAZOE, A.	Biochemical studies on the liquefaction of fish body - III. On effectiveness of fish-autolysate to the culture of bacteria	Ibid., **21** (12): 1226～1232 (1956)
TOMIYAMA. T., YONE, Y.	Studies on unidentified APF in liver - I Alleviative effect of a purified fraction of liver on the toxicity of pyridine 肝臓の未知動物蛋白因子に関する研究 Ⅰ. ピリジン毒に対する肝中の未知 APF の効果	Ibid., **22** (4): 257～259 (1956)
TOMIYAMA, T., ISHIO, S., KOBAYASHI, K.	Absorption of dissolved ^{45}Ca by *Carassius auratus*	Research in the effects and influences of the nuclear bomb test explosions (Tokyo): 1151～1156 (1956)
TOMIYAMA, T., ISHIO, S., KOBAYASHI, K.	Absorption by *Carassius auratus* of ^{45}Ca contained in *Rhizodrilus limasus*	Ibid., 1157～1162 (1956)

Tomiyama, T., Kobayashi, K., Ishio, S.	Absorption of dissolved ^{45}Ca by *Rhizodrilus limasus*	Research in the effects and influences of nuclear bomb test explosions (Tokyo) : 1173～1176 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Absorption of dissolved ^{45}Ca by marine fishes	Ibid., : 1163～1167 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Excretion of absorbed ^{45}Ca by gold fish	Ibid., : 1169～1172 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Excretion of absorbed ^{45}Ca by *Rhizodrilus limasus*	Ibid., : 1177～1180 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Absorption of ^{90}Sr (^{90}Y) by carp	Ibid., : 1181～1187 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Distribution and excretion of intramuscularly administered ^{90}Sr (^{90}Y) in carp	Ibid., : 1189～1193 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Absorption of $^{32}PO_4$ ion by carp	Ibid., : 1195～1200 (1956)
Tomiyama, T., Kobayashi, K., Ishio, S.	Distribution and excretion of intramuscularly administered $^{32}PO_4$ by carp	Ibid., : 1201～1203 (1956)
Tomiyasu, Y., Toyomizu, M., Takahashi, K.	On the deodorization of fishness by adding a ginger 生薑による魚臭の除去について	Nutrition and Food, 7(6) : 24～25 (1955)
Tomiyama, T., Nomura, M., Kuroki, S.	Effectiveness of aureomycin on keeping quality of sardine Aureomycin によるイワシの鮮度保持	Bull. Japan. Soc. Sci. Fisheries, **21** (4) : 262～266 (1955)
*Tomiyama, T., Kuroki, S., Nomura, M.	Keeping quality of mackerel, *Scomber japonicus*, by using aureomycin	Ibid., **21** (8) : 958～961 (1955)
Tomiyama, T., Kuroki, S., Maeda, D., Hamada, M., Honda, A.	Effectiveness of chlortetracycline (Aureomycin) on keeping quality of pacific round herring, *Etrumeus micropus* Aureomycin によるウルメイワシの鮮度保持	Ibid., **22** (2) : 120～127 (1956)

TOMIYAMA, T., etc.	Effectiveness of chlortetracycline on keeping quality of several bottom fish Aureomycin による底引漁獲物の鮮度保持	Bull. Japan. Soc. Sci. Fisheries, **22** (2): 128～135 (1956)
TOMIYAMA, T., YONE, Y., SUGAWARA, N.	Studies on the method for testing the spoilage of food VIII. A new turbidimetric method for determination of freshness of cooked fish-paste "Kamaboko" 食品の鮮度測定法に関する研究 Ⅷ. 蒲鉾の新鮮度測定法	Ibid., **21** (8): 954～957 (1955)
TOMIYAMA, T., YONE, Y., HARADA, Y.	Studies on method for keeping quality of "Kamaboko", a cooked fish-paste. I. Effectiveness of various preservatives, casing, infrared ray and ultraviolet ray, and their combinations. 煉製品の鮮度保持に関する研究 Ⅰ. 紫外線，赤外線包装及び塗布した防腐剤の効果	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (4): 551～564 (1956)
KAWABE, K., TOMIYAMA, T.	Studies on purification of industrial waste III. A critical study on amount of waste to be treated and of effluent permissible tobe discharged to the river. 工場廃水の浄化方法に関する研究 Ⅲ. パルプ廃液の酸添加処理限度並びに放流についての具体的考察	Bull. Japan. Soc. Sci. Fisheries, **20** (12): 1081～1085 (1955)
KAWABE, K., TOMIYAMA, T.	Studies on purification of industrial wastes - IV. Treatment of wasted bleaching liquor in paper mill 工場廃水の浄化方法に関する研究 Ⅳ. 製紙工場の漂白廃液について	Ibid., **20** (12): 1086～1088 (1955)
KAWABE, K., TOMIYAMA, T.	Studies on purification of industrial wastes - V. On the nature of the poisonous substance in alkali pulp waste 工場廃水の浄化方法に関する研究 Ⅴ. アルカリパルプ廃液の毒性について	Ibid., **21** (1): 37～41 (1955)
ZENITANI, B.	Yeasts occurring in fishery- fermentation products - VI On the cultural and physiological characteristics of asporogenous yeasts 水産醗酵食品の酵母に関する研究 Ⅵ. 無胞子酵母の培養的並に生理学的性質について	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (1): 53～61 (1955)

ZENITANI, B.	Yeasts occurring in fishery-fermentation products - VII. A taxonomic study of asporogenous yeasts Ⅶ. 無胞子酵母の分類学的位置について	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (1) : 63～69 (1955)
ZENITANI, B.	Yeasts occurring in fishery-fermentation products - VIII. On the origin and the significance of yeasts and bacteria in various "Shiokara" Ⅷ. 各種塩辛中の酵母及び細菌の来源及び意義について	Ibid., **15** (3) : 351～357 (1955)
TOMIYASU, Y., TOYOMIZU, M., TAKAHASHI, K.	On the existence of an antibiotic substance in "Katsuo- and Uni- shiokara" (Antibiotic oil and reversal action to it) カツオ及びウニ塩辛中抗菌性物質の存在について（抗菌性魚油とそれに対する阻害作用）	Sci. Bull. Fac. Agr. Kyushu Univ., **15** (1) : 47～52 (1955)
TOMIYASU, Y., TOYOMIZU, M.	Studies on the antibiotic action of fish components - VII. On the antibiotic action of the autoxidized calamary oil 魚体成分の抗菌作用に関する研究 Ⅶ. 酸化イカ肝油の抗菌作用について	Bull. Japan. Soc. Sci. Fisheries, **21** (4) : 253～257 (1955)
TOYOMIZU, M.	Studies on the antibiotic action of fish components VIII. Fractionation of an antibiotic substance from autoxidized fish oils Ⅷ. 酸化魚油中の抗菌性物質の分別	Ibid., **21** (4) : 258～261 (1955)
TOYOMIZU, M.	Studies on the antibiotic action of fish components - IX. The relation between the antibiotic action of the autoxidized shark liver oil and thiobarbituric acid value	Ibid., **22** (6) : 368～373 (1956)

—— Fishery Research Laboratory ——

TANAKA, O.	A new species of copepod, *Scolecithricella vespertina*, sp. from the Sea of Japan.	Records Oceanogr. Works Japan, **2** (1) : 117～120 (1955)
*TANAKA, O.	Rare species of Copepoda, Calanoidea, taken from the Izu region	Breviora, No. 64, 1～8 (1956)
TANAKA, O.	The pelagic copepods of the Izu region, middle Japan. Systematic account I. Families Calanidae and Eucalanidae	Publ. Seto Mar. Biol. Lab., **5** (2) : 251～272 (1956)
TANAKA, O.	The pelagic copepods of the Izu region, middle Japan. Systematic account II. Families Paracalanidae and Pseudocalanidae	Ibid., **5** (3) : 367～406 (1956)
TANAKA, O.	Further note on *Calanus tonsus* BRADY in Japanese waters	J. Oceanogr. Soc. Japan, **12** (2) : 49～52 (1956)

シダモクに関する二三観察[1)]

沢　田　武　男

Some observations on *Sargassum filicinum* Harvey

Takeo Sawada

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シダモクに関する二三観察[1)]

沢　田　武　男

Some observations on *Sargassum filicinum* Harvey

Takeo Sawada

褐藻ホンダワラ科のシダモク *Sargassum filicinum* Harvey は，従来アカモク，ヨレモク等と同じく subgen. *Bactrophycus* J. Ag. に置かれている植物で，その外観はアカモクと非常によく似たものである．ただ今迄のところ比較的稀なものとされていて，遠藤（1907）もそのように記している．又同博士の図解には生殖器托，葉及び気胞の夫々一ケ宛を有する枝の一部が示してあるにすぎない．岡村（1936）の“日本海藻誌”にも冒頭に「未だ全形を知らず」とあり，二三の性質に就いて簡単に記してあるだけである．この種の原記載を行つた Harvey（1859）の材料も不完全であつて sterile なものであつたようである．従つてシダモクに関しては今迄のところ相模，常陸の採集地とその外形に関することだけが報告されていて，これとよく似たアカモク，トゲモク等との類似点，相異点が知られているのみである．

最近，肥後・長門・伊豆・房州等の各地でシダモクを打揚げその他によつて，充分とは云えないが若干の材料を入手出来た．特に肥後・伊豆の材料によつて観察を行つたところ，二三興味ある事実を知ることが出来たので報告したい．

本文を草するに当り懇切なる御指導と御校閲を賜つた九大農・水産学教室・瀬川宗吉助教授並びに始終御激励と御便宜をいただいている内田恵太郎教授に深謝する．又材料入手の上に種々御便宜をいただいた東教大・下田臨海実験所・千原光雄理学士並びに東邦大・理・大久保真理子氏に謝意を表する．

I. 成　熟　期

アカモクの成熟が2月頃から既に見られるのに較べ，この種の成熟期は相当晩いと思われる．このことは入手した材料中，3月末に採集した肥後の標本では全く未熟で未だ生殖器托を付けておらず，8月末の伊豆・房州のものでは生殖器托は充分熟しており，既に末期に近いものであつたことから想像される．即ち大体晩春から8～9月にかけて熟するものと思われる．

II. 外　　形

外形の最も著しい特徴は気胞が球形をなしていることで，気胞以外は小造りではあるが

1）日本植物学会第19回大会（昭29.10, 於京都）にて発表．

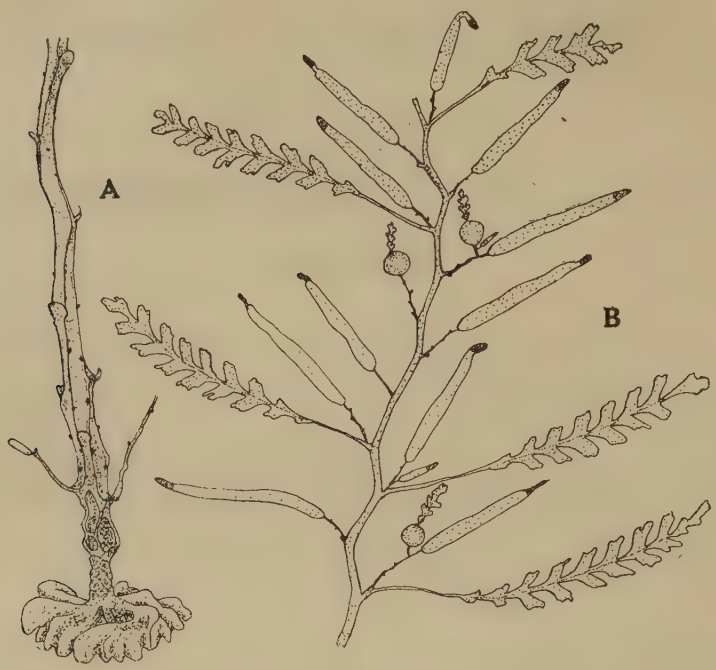

Fig. 1. Basal part (A) and a branch (B) of *Sargassum filicinum* Harvey (×4/5).

アカモクと非常によく似ている (Fig. 1). 即ち根はアカモクと同様に仮盤状根であり，茎は単条でその稜角に刺状突起を持つている．各方面に枝を分ち，全長1m 時に 1m 半にも達する標本も得られた．

気胞は前述の通り球形であるが，冠葉は葉と似ており，又アカモクのものとも酷似している．生殖器托の円筒状なることもアカモクの場合と同様である．

III. 生 殖 器 托

生殖器托はアカモクの雌生殖器托に近い外形をなし，柄によつて枝に付いており，孤立する場合もあるが又すぐ近くに可成り小形の生殖器托を伴つている場合もある．一般に長さは 2.5cm 前後，太さ2～3mm のものが多いが，長いものでは 4.5cm 以上のものもあり，短いものでは 2～3mm ばかりのものまである．

アカモクその他多くのホンダワラ類とは異つて，雌雄両生殖窠は同株且つ同一生殖器托上に生ずることは特記すべきことである．又このことは ハハキモク 及び ***Eusargassum*** 亜属のものでよく見られる例であるが，これらとも異つて注目すべきは雌雄生殖窠が生殖器托上に場所を違えて生ずることである．即ち雄生殖窠 (Fig. 2, B; Fig. 3, A) は生殖器托の基部の附近にのみ生ずることである．普通大の生殖器托に於ては雄生殖窠はその基部1割前後の少部分にのみしか存在せず，中央部や先端部からは検出されなかつた．又雌

生殖窠（Fig. 2, A）は中央部や先端部だけに存在し，下部の雄生殖窠の部分には見られなかつた.[1] ただ雌雄の境界の部分にあつては，横断面に於て雌雄両生殖窠の混在が見られた.

ホンダワラ属及びその近縁属の，雌雄同株で雌雄両全の生殖器托を有するものに就いて考えてみると，(1) ヒバマタ等のように生殖窠として雌雄同窠のものと，(2) *Cystoscira* によく見られるように同一生殖器托上に雌雄同窠のものの他，雌並びに雄時にその一方の生殖窠も見られるもの（Sauvageau, 1912）及び (3) 雌雄異窠のものが存在する．(3) の場合は即ち雌雄同株，雌雄同生殖器托，雌雄異窠であつて，従来ハハキモク及び *Eusargassum* 亜属の諸種等で知られていた（Grunow, 1915, '16；山田，1942；猪野，1947）．これにシダモクの例が加わるわけであるが，上述の特徴によつてこれらの種とは著しく異なる型となる．このような型を示す記述としては Kützing（1860）がアカクモの条下で，体の一部を示した図（Tab. Phy., **10**, 31, t. 89, f. 1, c）の説明として "Bruchstück eines andern Exemplars, dessen Fruchtkörper in der obern Hälfte Fruchthöhlen mit Sporen, in der untern

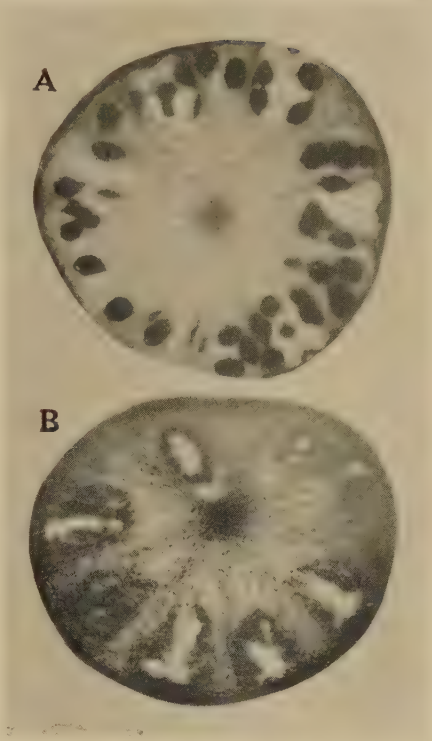

Fig. 2. Transverse sections of the receptacle of *Sargassum filicinum* Harvey.
(A) Female part (×20).
(B) Male part (×20).

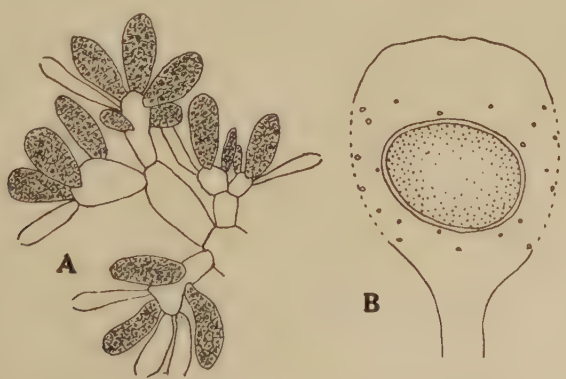

Fig. 3. Antheridia (A) and a discharged oosphere (B) of *Sargassum filicinum* Harvey (A,×450; B,×65).

1）尚この事実は東大，理，植物学教室保存の遠藤博士のシダモク標本（Kawajiri, Hitachi, Aug. 1907. Coll. M. Tago）に於ても確めることが出来た．この観察に関し特別の御便宜を供与された前川助教授に謝意を表する.

aber Fruchthöhlen mit Antheridien enthalten, in natürlicher Grösse." の如く記している.[1] Kützing の図によれば，この観察の材料になつたものは気胞が球形ではなく魚雷型で，アカモクに違いないと考えられるものである．何れにしてもこのような性質がアカモクでも表われることがあるか否か検討を要する．

IV. 卵 放 出

前述の如く普通大の生殖器托に於て，上部の9割前後は雌生殖巣の部分と考えられるのであるが，他のホンダワラ類と同様（田原，1913; etc.）雌生殖巣は下部から次第に上部に向つて熟して行き，卵の放出も少部分宛3～4回位に行われるのではないかと考えられる．このことは材料に放出中のものがあり，その放出部分の長さから推してそのように考えられる．

放出卵を観察した材料は8月末のものであつて生殖器托としては成熟終期に近く，先端に近い部分で放出されており，膠質状物質によつて生殖器托表に留まつているのが見られた．尚，小形の生殖器托では全表面に渉つて見られる場合もあつた．

須藤（1948）はホンダワラ類の受精に就いて数種の材料により観察した結果，精子の放出卵への侵入位置は種によつて予め定まつており，放出卵を包む蔵卵器中膜の膨潤部から穿入して卵に近ずくことを報告している．アカモクではこの膨潤部が他の種に比して特殊で，帯状に卵を取り巻いている事実が確められた（国枝・須藤，1940; 須藤，1948）．今回シダモクに就いても同様に卵を帯状に取り巻く膨潤部のあるのを観察することが出来た（Fig. 3, B）．

受精卵の其の後の発育段階を示すと思われる材料は見当らなかつた．従つて仮根細胞の分裂型及び第一次仮根数は確められなかつた．しかしアカモクの放出卵に比して可成り近い大きさであつた点から考えると，猪野（1947）の仮根数と卵の大きさに就いての意見から推して，仮根細胞の分裂型はアカモクと同じく放射8細胞型か16細胞型，第一次仮根数は16ではないかと云う想像が許されよう．このことに就いては今後材料を得て観察究明したいと思う．

摘 要

1. 著者は従来不明な点の多かつたシダモクに就いて肥後・長門・伊豆・房州の材料，特に肥後・伊豆のものに就いて観察し，二三興味ある知見を加えることが出来た．

2. 成熟期はアカモクに比して晩く，晩春から8～9月の間と思われる．

3. 外形は気胞が球形であると云う相異点を除き，根・茎・葉・生殖器托等は小造りではあるがアカモクに非常によく似ている．

4. 生殖器托は普通大のものに於て外形も大きさも大体アカモクの雌生殖器托に似てい

1）これに対して遠藤（1907）は同じくアカモクの項で Kützing の説明の不適当であることを指摘してゐるが，同博士の考察にも又考え違いがあるようである（詳細略）．

る．しかし異なる点は雌雄両生殖巣は同株且つ同一生殖器托上に生ずることである．更に，生殖器托の基部1割前後の少部分には雄生殖巣のみ，その上部9割前後の部分には雌生殖巣のみが存在することも特異である．

5. 卵の放出は他のホンダワラ類と同様，下部から上部に向つて少部分宛行われるものと思われる．

6. 放出卵は他のホンダワラ類と同様，膠質状物質に包まれているが，アカモクと同様に卵を取り巻く帯状の膨潤部が観察された．

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Résumé

In this paper the writer intends to make some interesting remarks on *Sargassum filicinum* regarded as a comparatively rare species.

In 1954, the writer was able to recieve two sets of material of this alga, one from Higo Province in the last part of March and the other from Izu Province in the last part of August. The former material is very young and has no receptacle yet; the latter's receptacles are in the later stage in mature. From these facts it seems that the alga is in mature during the summer.

With the exception of having the spherical vesicles the alga bears a strong resemblance to *S. Horneri* in appearance: i. e., the disco-fibrous root, the fine spinous processes on stem, the cylindrical receptacles and the leaves are almost equal to ***S. Horneri.***

Receptacles also resemble the female ones of *S. Horneri* in external shape, but they are entirely different from the latter by being androgynous. In each receptacle of moderate length, male conceptacles are found only in the basal part within about one tenth of the receptacle. In the upper part excepting the basal male part only female conceptacles are found.

Discharge of oogonia takes place in a girdle around the receptacle, and it seems that this discharged portion advances acropetally in each receptacle as in many other members of the genus *Sargassum.*

The discharged oosphere is surrounded by a thick mucilaginous substance around whose outer side there is observed a girdle-like disolved portion very like as in the discharged oosphere of *S. Horneri.*

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Kyushu University

オゴノリの果胞子放出に関する研究

第1報 蔭干に伴う果胞子放出について

瀬川宗吉・尾形英二 ・沢田武男

Studies on the carpospore liberation in *Gracilaria verrucosa* (Huds.) Papenfuss

I. Carpospore liberation accompanied with the half-drying

Sokichi Segawa, Eizi Ogata and Takeo Sawada

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オゴノリの果胞子放出に関する研究[1]

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瀬川宗吉・尾形英二[2]・沢田武男

Studies on the carpospore liberation in *Gracilaria verrucosa* (Huds.) Papenfuss[3]

I. Carpospore liberation accompanied with the half-drying

Sokichi Segawa, Eizi Ogata and Takeo Sawada

I. 緒　　言

有用な水産植物の増殖にあたつて近時積極的にタネ（胞子）付けが考慮されるに到つた（須藤，1948 etc.）．この場合まず問題になるのは，如何にして多量の，しかも有効なる胞子を短時間内に得るか，と云うことである．この様にして得た胞子を出来得る限り最少の損耗をもつて養育するには如何にすべきかと云う問題がこれに続くと思われる．

一時に多量の胞子を得る方法の一つとして蔭干法が考えられる．即ちタネクサ採取後幾時間か湿つた冷所に置いた後海水に浸けて胞子を放出させる方法であつて，既にワカメに於いては木下（1944）に依つて研究され実用化されている．又紅藻に於いても須藤（1949）のフノリに関する報告があり，これも既にある程度実用化されている．

しかし胞子放出の基礎的事項に到つては決して研究しつくされたわけではなく，不明な点が相当にあり，随つてその実用化にあたつて充分なる計劃性を持ち得ない．このことは不動胞子しか持たない紅藻で特に強く考えられるところである．

そこでこれら基礎的な事項をまづオゴノリを材料として，果胞子放出についての研究から開始した．材料としてオゴノリをとり上げたのは紅藻嚢果の代表的な型の一つを持つていることと，他方現在オゴノリの需要が高まりつつあることからである．

その第1報としてまづ蔭干に伴う果胞子放出を取扱つた．一般に潮間帯に棲処を持つている藻類は多く干潮の際胞子放出を行うと思われている（須藤，1950）．オゴノリは潮間帯の下位に多く生育しているので毎日干出するわけではないが，月の何日かは干出する個体が多い．したがつて干潮に際して胞子放出が行われることは当然想像されるところである．その際に於ける放出の模様は蔭干処理に伴う放出と似ていると思われる．そこでその放出の全経過並に胞子の性質について吟味して見た．

1）文部省科学研究費による「紅藻胞子の放出に関する研究」の一部．

2）大阪市立大学理工学部．

3）従来 *Gracilaria confervoides* (L.) Grev. とされて来たものの変更名．Papenfuss (1950) 並に近江 (1954) 参照．

II. 材料及方法(概要)

材料として福岡湾東部の多々良川口附近に産する嚢果を有する個体を用いた. 時季は主として6月中旬から8月上旬迄で当地方オゴノリの繁殖最盛期にあたる. 実験は 1952 年実施した.

採集は主として退潮時に行つた. 海水に浸つているものを選び, 干出して了つたものは避けた. 採集した材料は胴乱又は魚籠に入れ自転車で約 10 分の距離にある実験室に持ち帰り, そのまま涼しい湿気ある流しに置いて2～92時間蔭干の後実験に供した. この際蔭干時の室温は 21～31° C であつた. 生育地の水温は採集の都度測つたが 22～27° C の範囲であつた.

尙材料及方法に関する詳細は後述各項にゆづることとし, ここでは概要にとどめる.

III. 嚢果からの胞子放出の全経過

この実験は1嚢果から1回の胞子放出が行われる際, 如何なる経過をとるかを明かにする目的を以て行われた. この様な観察はいくつかの紅藻に於いて既に Chemin (1937) によつて行われたのであるが放出全経過としての観察は不充分であつた. 又オゴノリに関しては既に瀬川・右田 (1951) によつて大体同様の研究結果が報告されていたのであるが今回は更に組織的に追試してみた.

A. 材料及方法

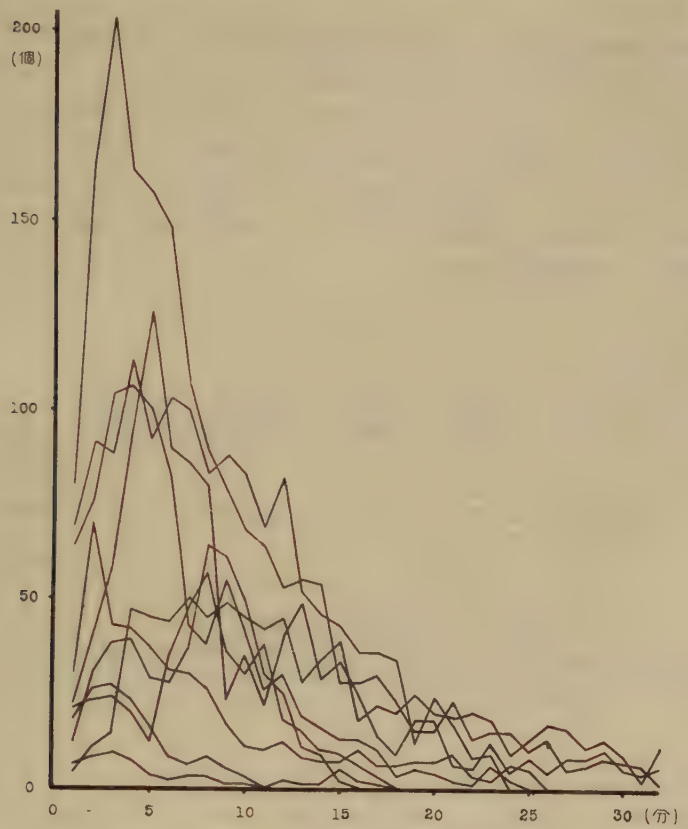
濾過海水を浅く入れたシヤーレを顕微鏡下に置き, 前述した材料の枝を適当の大きさに切つてその中に入れて鏡検すると, やがていくつかの嚢果が胞子放出をはじめるので, そのうちの1嚢果について放出の全経過を観察記述した. 実験は極めて多数の材料について何回も行つた.

B. 結果

材料を海水に浸してから放出を見るまで0～10分, 多く数分の間がある. まづ放出に先立つて嚢果の果口から微粒を含んだ粘液が吐出される. ついでその粘液の流れの中に胞子が含まれる. 必ず1個宛放出されるが2～4個宛多少相続いて放出されることが多い. 果口並に果溝が狭隘な場合胞子はアミーバ状の変形を受けるが, 果口を離れると楕円—卵形となり前述の粘液の糸に連つて漸次に沈下して行き底に着いてから円くなる. 尙放出中には内容の色が淡くて未熟と思われる胞子や胞子以外の異物が混つていることもある. 特に放出の末期に多い.

材料の枝に着いている嚢果がすべて放出を行うことは殆どなく, 全然放出を行わない嚢果も多い. この様な嚢果は外貌から多少見当をつけることも出来るが, しかし殆ど同じ外貌を持ちながら一方は放出を行い, 一方は全然放出を行わない嚢果が相隣つている場合も見られる.

単位時間(1分)の胞子放出数が時間の経過と共にどの様に変つて行くかについての10例を第1図に示した. この様な多数の実験から大凡そ次の様なことがわかつた.



第1図. 時間の経過に伴う放出胞子量の変化.

(a)　1回の放出は10～50分，時に80分を要する．もつとも放出の終了を見極めることは中々困難な場合があり，この実験では2分間放出を見なかつたことを限度として観察を打切り，その時を終了と見做した．

(b)　1嚢果から1回に放出される胞子数は嚢果によつて大差あり，200～2000 個に渉る．時に 4221 個の場合があつた．

(c)　単位時間に対する放出数は放出初期に多く最高値もそこに見られる．中期後期と漸次減少して終了となる．

以上の3事項のうち放出時間，放出胞子数は同一処理の材料でも嚢果によつてかなりの差が見られる．

IV.　放出の前後による胞子の性質の差異について

この実験は1嚢果に於ける1回の放出の前，中，後期によつて放出された胞子の性質に差異が在るか否かを大きさ，破壊率及活力について比較してみた．

A. 材料及方法

7月下旬の II 項に述べた様な材料を長さ5mm 位の小枝としその体上に1個の嚢果のみを残した．次に海水を入れたシヤーレの中にスライド硝子を入れ，この上に硝子の小リングを並べて置き，放出を始めた材料をその中に入れて3～5分位宛順次にリングを変えて放出させ，放出が終るまで続けた．この様にして放出胞子はリングの中に落下し他の時期のものと混らない様にスライド硝子に附着させた．実験は多く夕刻行つたが大きさは放出後 10 分位経つた時測定した．破壊率及活力などは翌朝又は翌々朝調べた．即ち経過時間は約 15 時間又は 39 時間であつた．

B. 結　果

(1) 胞子の大きさ

第1表にその結果を示した．放出を前中後の3期に分ち，各期の胞子を 600 倍の倍率で鏡検して，無作為にえらんだ5視野（径 300 μ）の胞子の直径を測定し比較値の材料としたものである．

これによつて大凡そ前期に大きいものが多く，中期と後期は小さく大差ないことが示されている．

第1表．　放出の前後による胞子の大きさ．

胞子数 ＼ 大きさ μ	21.75	23.40	25.05	26.70	28.35	30.00	31.65	33.30	平均値(μ)信頼度 95%
前　期	—	—	5	5	10	15	1	1	28.5 ± 0.7
中　期	4	6	4	11	—	—	—	—	24.8 ± 0.7
後　期	3	6	5	9	4	3	—	—	25.8 ± 0.9

(2) 破　壊　率

胞子中には発芽をすることが出来ず内容を吐出して破壊するものが見られた．第2表はその結果の4例を示したものである．破壊数の計数には任意の1視野中のものを数へて代表値としたものがある（*印）．

第4例を除き破壊率は明かに後期になるにつれて高くなつていることがわかる．

第　2　表．　放出の前後による各時期胞子の破壊率．

第1例　7月22日採取

時間（分）	0	4	8	12	16	20	24	28	32	36
放出胞子数	487	365	98	82	51	33	38	19	6	
破壊数	1	5	4	0	0	0	7	6	1	
破壊率(%)	0.2	1.4	4.1	0	0	0	18	31.6	17	

第2例　7月25日採取

時間(分)	0	5	10	15	20	25	30	35
放出胞子数	446	132	47	42	17	15	1	
破壊数	6/65*	6/79*	9/32*	9/30*	17	15	1	
破壊率(%)	9.3	7.6	28	30	100	100	100	

第3例　7月28日採取

時間(分)	0	5	10	15	20	25	30
放出胞子数	505	166	56	34	18	15	
破壊数	4/ 90* 12/170*	4/34* 6/88*	5/29*	4/12*	5/14*	4/7*	
破壊率(%)	4.5 1.4	12 6.8	17	33	36	57	

第4例　7月31日採取

時間(分)	0	4	8	12	16	20	24	28	32	36
放出胞子数	368	220	185	76	41	36	18	5	18	
放壊数	50	4	39	31	13	2	0	1	5	
破壊率(%)	13.6	1.8	15.7	41	32	5.6	0	20	28	

(3) 活　力

発生をはじめた発芽体の間にはその発育速度に遅速があつて，外見上細胞分裂が早く行われるもの程活力が旺盛な様に見える．そこで発芽体の分裂細胞数を数えた結果の3例を第3表に示した．

これによつて明かに前期のものは早く細胞分裂を行つて細胞数が多くなつて居り，後期のもの程少いことがわかる．しかし第3の例では後期に放出されたもので細胞数の多いこともあつた．

第　3　表．　放出の前後による各時期胞子の活力(分裂した細胞数で示す)．

第1例　7月22日採取

時間(分)	0	4	8	12	16	20	24	28	32	36
放出胞子数	487	365	98	82	51	33	38	19	6	
細胞数	10—20	8—18	8—18	8—16	2—15	2—15	1—15	1—15	1—10	
最も普通な群の細胞数	14—15	10—15	10—15	10—15	10—12	10—12	8—10	6—8	4—10	

第2例 7月28日採取

時 間 (分)	0	5	10	15	20	25	30
放出胞子数	505	166	56	34	18	15	
細 胞 数	1—20	1—10	1—10	1—4	1—2	1—2	
最も普通な群の細胞数	15—16	10	4—8	2—4	1—2	1—2	

第3例 7月31日採取

時 間 (分)	0	4	8	12	16	20	24	28	32	36
放出胞子数	368	220	185	76	41	36	18	5	18	
細 胞 数	2—16	1—16	1—16	1—12	1—12	1—12	1—4	1—5	1—16	
最も普通な群の細胞数	8—14	1—12	1—12	8—10	1	1	1	4	4—10	

V. 嚢果内に残つた胞子並に放出を行わなかつた嚢果内の胞子の性質

放出を了つた嚢果内には外見上未熟胞子と思われるものの他に尚放出胞子とあまり変らない胞子も多数包蔵している. 又 III 項にて述べた様に同処理をしても放出を行わない嚢果内にも放出嚢果と同様未熟胞子の他に未熟とも完熟とも判断出来ない胞子を蔵している. これら嚢果内の一応未熟として取扱うべき胞子の性質については既に沢田 (1954) が断片的に予報したのであるがここでその結果の詳細を述べる.

A. 材料及方法

7月下旬採集した材料で III 項の実験と同様蔭干後浸水して放出を行つた嚢果①, 並に放出を行わなかつたが外見上①とあまり変らない嚢果②を対象とした.

海水を入れたシヤーレに材料を入れ, 解剖顕微鏡で鏡検し乍ら, ①及②の果皮を解剖針で圧して出させた胞子, 並びに果皮をとりはずし嚢果内から摘出した果胞子群について別々に培養し, その経過特に破壊率並に分裂細胞数の増加を観察した. 前実験と同じく夕刻実験を行い翌朝 (約 15 時間経過) 調べた.

B. 結 果

破壊率及分裂細胞数を①及②について2例宛第4表に示した. * 印は前実験と同様, 任意の1視野について計数したものである.

この表によつて明かな様に①及②の胞子でも機械的に出して培養すると (破壊率は高率を示した例もあるが) 結構発育をはじめ, 特に圧し出した胞子に於いてはその分裂細胞数が放出された胞子と匹敵するものさえあることがわかつた. 尚それら発芽体は実験を終了するまで5日間の発育を続けた.

第4表.　機械的に囊果から出した胞子の破壊率及び分裂細胞数で示した活力.

			胞子数	破壊数	破壊率(%)	細胞数
① 放出後のもの	第一例	1	120	1/38*	2.6	15—16
		1′	87	2/47*	4.3	15—16
		2	仁	若干	—	1—10(4が多い)
	第二例	1	23	11	48	1—4 (殆ど1)
		2	仁	約半数	ca50	1
② 放出のなかつたもの	第一例	1	159	33	21	15—20
		2	仁	21/125* 24/133	17 18	1—8
	第二例	1	168	34/118*	29	10—20
		2	仁	28/85*	30	1—4(殆ど1)

1 ：果皮を圧して出したもの.　1′：1でまだ残つたものを強く圧して出したもの.
2 ：仁を摘出したもの.

VI. 要　　結

本報に於いてはまず最初に蔭干処理後の浸水によつておこる果胞子放出の詳細を記述したが，この様にして実験室内に於いて観察された放出の模様は，それが全く同様ではないにしても干出後の上潮時にもおこつているらしいことは，別に野外の小実験に於いても確めている．更に比較実験を繰返してこの点を確実にしたいと思う．

放出の際果胞子に伴う粘液の流れは果腔内の隙間を満している物質か又は細胞膜間物質の膨潤によるものと思われるが確言は出来ない．しばしば混入して来る異物は nutrient tubular cell (Sjöstedt, 1926) の破片とも思われる．

放出は浸水後 0 ～10分，多く数分の間をおいておこり，10～50 分位で終了し，200～2000 個も放出される．1囊果から1回に放出される胞子の数は他の紅藻に比して甚しく多いと思われる．

放出量は初期に多く中期後期と漸次減少して終了となる．この事実は蔭干後の浸水によつて囊果の放出能力が急に賦与された結果，前期の山となつてあらわれ，その後その力が漸次に減衰するため中期後期の漸減を来すと思われる．囊果は蔭干以前の状態と全く等しい状態に復帰するとすれば，紅藻胞子は一応他動的と考えられるからこの力は生じないことになる．即ち放出囊果は吸水によつて蔭干処理以前と異つた状態になるのである．これについて考えられることは囊果の構成部分である果腔を囲る組織（果皮を含む），果胞子群，並にそれらの隙間を満している物質等の膨潤の度合に不均衡を生じた結果の内圧のためと思われる．この様な放出の機作に関してはある程度の知見を得ているのであるが後報にゆずる．

同じ処理を受けた囊果に於いて，放出したものと外形も殆ど差異がないのに放出のおこらぬ囊果がよく見受けられる．この場合その果内には一見未熟とは思えない果胞子が含ま

れている．この様な嚢果は蔭干処理を受けても可逆的に元とほぼ等しい状態にもどつたものと考えられる．

次に本報では放出の時期による胞子の性状の差異を取扱つた．放出の前期に於いて出る胞子の径は大きく．破壊率も低く，生活力も旺盛であるということは考えられることであるが，中期後期のものが一般に径も少し小さく，破壊率も高く，生活力も劣つている傾向を示すのは蔭干効果が過ぎて自然には出ない胞子までも放出されるためとも考えられる．更に研究を要する．

放出嚢果並に不放出嚢果の果内に残つた胞子で特に圧して出て来る胞子がかなりの発芽能力を持つていることは注目すべきことと思われる．ただしこの場合附着力その他の性質が如何様になつているかは更に研究を要する．

終にのぞみ，この研究に種々便宜を与えて下さつた内田恵太郎教授に御礼を申上げる．尚この研究の初期に種々協力していただいた長崎大学水産学部右田清治講師に謝意を表する．

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Summary

In the field the carpospore liberation from the cystocarp in *Gracilaria verrucosa* seems to occur chiefly when the tide is rising as reported by Suto (1950) in *Gloiopeltis*. The details of such liberation were investigated in the laboratory. The materials were collected in Hakata Bay in their maturating season: June to July. After half-dried for 2–92 hours, the materials were immersed in sea water for observation.

After 0–10 minutes, some of their cystocarps begin to liberate carpospores one by one. About 200–2000 carpospores are liberated once from each cystocarp, taking about 10–50 minutes. In the early stage the spores are liberated in a large number, and the number gets thinner towards the later stage.

The spores liberated in the middle and later stages are slightly smaller than in the early stage. The broken spores were observed in a considerable number in culture of the spores liberated in the later stage.

As to the germination, the spores which remain in the cystocarp after liberation and also in the one without the occurrence of liberation, were examined after they were pushed and then picked out of the cystocarp. The obtained result was that the larger number of such seemingly immature spores were also able to germinate as in the liberated spores in culture.

オゴノリの果胞子放出に関する研究

第2報　果胞子放出の機作について

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オゴノリの果胞子放出に関する研究[1)]

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I. 緒　　言

第1報（1955）においては，上潮にともなつておこると思われる果胞子放出の過程を，実験室内で蔭干法によつて明かにした．しかし，この研究の最終目的であるところの，どのようにして多量の有効胞子を短時間内に得るかという問題に対する基礎研究としては，これらの現象を知るだけでは充分でなく，更に放出の機作を解明する必要があると思われる．そこで同じオゴノリの嚢果を対象として，果胞子放出の機作に関する実験を行つた．

放出の現象のみに関する研究は，第1報緒言に述べたようにかなりみられるが，放出機構にまでふれた研究は殆どみあたらない．褐藻では，Schreiber (1930) が *Fucus serratus* について，・中沢 (1953) が *Coccophora Langsdorfii* について，外液の濃度が低まると生殖窠からの卵細胞の放出が促進されることを示唆している位である．この熟した藻体を海水の濃度が低い方へうつしたとき，ほとんどの場合放出が促進される現象（低張効果）は，著者等が数年来の実験で気がついていたのであるが，今度の研究にあたつて，この現象と蔭干効果との関係を考えてみた．

蔭干効果にしても低張効果にしても，共通な要因として第一に，藻体と外液の滲透圧の相対的変化が考えられ，従つておそらくこのことが放出促進の機作として重要なものと考えられる．この仮定をたしかめるために，濃度のことなる海水および数種の電解質・非電解質の溶液に藻体をひたしたときの，果胞子放出におよぼす影響をしらべた．

II. 材料および採集方法

第1報と同じく，博多湾東部の多々良川口附近に産する，完熟していると思われる嚢果を有するオゴノリを用いた．実験は，1953年の6月に行つた予備実験にもとずいて，1954年の6月から7月にかけて行つた．材料の採集にあたつては，主として退潮時にまだ海水

1）文部省科学研究費による「紅藻胞子の放出に関する研究」の一部．

2）大阪市立大学理工学部．

にひたつているものをえらび，海水につけたまま研究室にもちかえつた．そしてもちかえつた直後，既知濃度の海水（比重：ca 1.017，滲透圧：15.9 atm. 20° C）にうつし，30分以上放置して滲透圧的に平衡になつてから実験に使つた．オゴノリの生育する海域には海水滲透圧の日変化があるが，その影響をふせぐためにほぼ同じあるいはやや低い滲透圧をもつときをえらんで採集した．

III. 実 験 方 法

1） 嚢果の放出率算定法

嚢果体の枝を 3 cm 内外に切り，ついている嚢果（約 10～25）のうち，処理によつて放出をおこした嚢果数の%を算定した．処理してから算定までの時間は，第1報の結果から20分を適当と考え，すべて20分後の放出率を求めた．用いた枝は，一つのシリーズの実験においてはなるべく同一個体から切りとるようにした．以後の実験値は，特にことわらない限り，すべて3回以上の測定の平均値である．

2） 用 い た 溶 液

用いた電解質・非電解質の溶液は，第1表のように海水を含めて6種である．これらの各に濃度の段階をつけて，その滲透圧が用意した海水（以下基準海水とよぶ）を基準として，それぞれ 2.0, 1.5, 1.0, 0.5, 0.25 倍になるように作つた．たとえば海水の2倍の滲透圧をもつ sucrose 液ならば，2.0 sucrose と以下記述することにした．なお滲透圧は氷点降下法で計り，Walter (1936) の補正法により気圧であらわした．

Table 1. Osmotic pressure (atm.) of different solutions used in experiments.

Osmotic Conc. / Solutions	2.0 fold	1.5 fold	1.0 fold	0.5 fold	0.25 fold
Sea Water	37.5	23.9	15.9	6.3	3.8
KCl	34.6	18.9	17.2	7.2	4.1
NaCl	36.9	18.9	17.1	6.6	3.9
$CaCl_2$	38.9	18.8	17.4	6.9	3.9
Sucrose	38.9	—	17.2	6.6	3.7
Glycerol	35.6	—	17.4	6.7	3.8

IV. 実験ならびに結果

1. 外液の滲透圧およびその溶質の差による影響

材料を基準海水から小型シヤーレ中の各溶液（第1表）にうつして静置させ，20分後に上記の方法で検鏡測定した結果は第1図のようである．

この図からわかるように，生育している所の海水と等張な電解質・非電解質中で，ほぼ sucrose, glycerol, KCl, NaCl の順で放出が促進され，$CaCl_2$ 中では放出がみられない．等張海水中では当然みられない．高張液中では一般に放出がおこらないものと予想さ

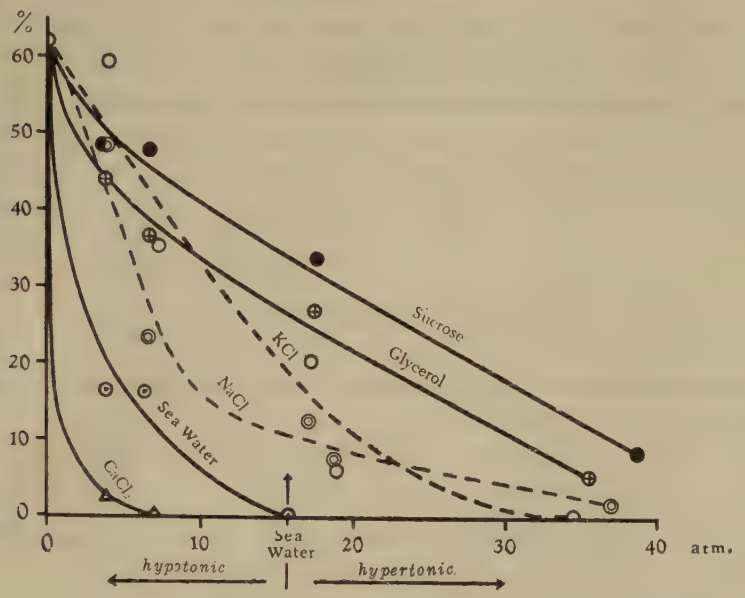

Fig. 1. Ratio (%) of spore-shed cystocarp in the different osmotic concentration of external solutions. (20 min. after immersion).

れていたが，2.0 sucrose, 2.0 glycerol, 2.0 NaCl 等では僅かであるが放出がみられる．

低張液中では更にこの傾向がつよまり，滲透圧が低くなるにつれて放出嚢果数の%が増す．なお，低張になるにつれて，%だけではなく1個の嚢果からの放出果胞子数も増す傾向があるようにみえる．$CaCl_2$ は低張液中でさえ，放出を抑制する作用がある．

海水中では予想されたとおり，高張側で放出せず，等張海水を出発点にして低張になるに従つて順次放出が促進される．

この結果からみて，すべての溶液を通じて低張になるほど放出を促進する傾向がみられるが，溶質によりその作用の程度がことなり，大体 sucrose, KCl, glycerol, NaCl 等はこの低張効果の他にそれら溶質独自の促進作用がみられる．$CaCl_2$ では逆にいちじるしい抑制作用がみられる．

2. 蔭干の影響

蔭干をした藻体を，もとの海水にもどすことによつておこる放出の促進現象については第1報のとおりであるが，この蔭干処理を実験1の処理の前に加えた場合どのような影響があるかをみた．

蔭干の条件を一定にするために，滲透圧が約 37 気圧に相当するような関係湿度をもつ恒湿室を硫酸によつて作つた．すなわち関係湿度が約 96.5 % になるように 9.38 % 硫酸をデシケーター中に入れて密室を作り，実験1のようにして用意したオゴノリの枝を，この中で一定時間乾燥させた．

Table 2. Ratio (%) of spore shed cystocarp in the different osmotic concentration of external solutions after desiccation for 20 min. (20 min. after immersion).

Osmotic Conc. / Solutions	2.0 fold	1.5 fold	1.0 fold	0.5 fold	0.25 fold
Sea Water	0.0	13.3	20.0	41.7	44.4
KCl	5.0	37.5	62.5	60.0	55.6
NaCl	0.0	6.3	25.0	45.5	30.8
$CaCl_2$	0.0	0.0	0.0	11.1	15.4
Sucrose	10.0	—	33.3	60.0	70.0
Glycerol	16.7	—	33.3	50.0	55.6

上記デシケーター中で 20 分蔭干した材料を実験1の場合と全く同様にして第1表の各溶液にうつし，やはり 20 分後の放出嚢果数の % を算定した（第2表）．この結果をみると，蔭干をしないで直接に基準海水から各溶液にうつした場合よりも放出が促進されているように思われる．すなわち蔭干による影響があるようである．

3. 拮 抗 現 象

低張でも $CaCl_2$ は放出に対して抑制的であり，逆に高張でも KCl; NaCl や sucrose, glycerol は促進することから，Ca^{++} と他の溶質間に拮抗現象があるかどうかをみてみた．実験はすべて 0.5 の $CaCl_2$ と KCl, $CaCl_2$ と NaCl, $CaCl_2$ と sucrose の間の作

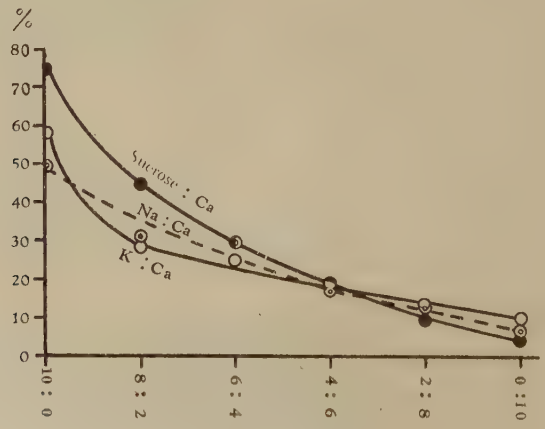

Fig. 2. Antagonistic effect between pairs like KCl and $CaCl_2$, NaCl and $CaCl_2$, sucrose and $CaCl_2$ on the ratio of spore shed cystocarp when algal bodies are transferred from natural sea water (15.9 atm.) to the hypotonic solutions (6.3—7.2 atm.) (20 min. after immersion).

用をみるために，$CaCl_2$ と他の溶液の割合を 10 : 0 から 0 : 10 までの間，6段階に混合した溶液を作った．それらの溶液はすべて同じ気圧（6.3～7.2 atm.）をもつ．処理は実験1と同じように行い，やはり 20 分後の影響をみた（第2図）．これをみると $CaCl_2$ の割合がますにつれてしだいに放出が抑制され，あきらかに Ca^{++} と他のものとの間の拮抗現象がみられる．イオン間だけではなく Ca^{++} と sucrose の間にもこの傾向がみられる．図にみられるように，この実験における Ca-effect は高等植物で多くみられる傾向とちがつて，効果のあらわれが緩慢である．

4. 高張液の浸漬処理による放出促進

低張液にうつした場合，つねに放出が促進されることは滲透圧の変化にともなう膨圧の

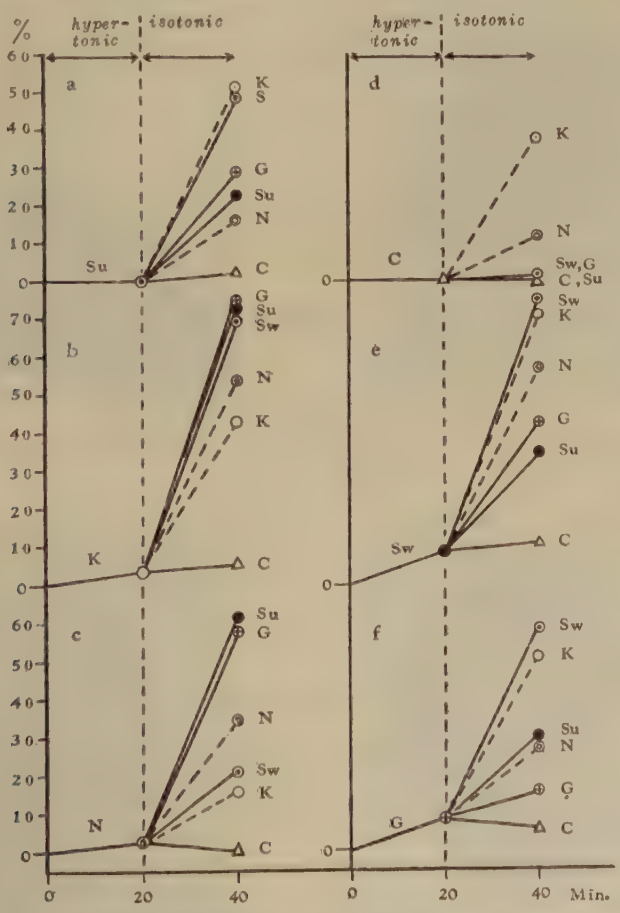

Fig. 3. Effect of different solutes on the ratio of spore shed cystocarp when algal bodies are transferred from the hypertonic solutions (2.0 fold) to ones isotonic with natural sea water (1.0 fold).

Sw : sea water, K : KCl, N : NaCl, C : $CaCl_2$, Su : sucrose, and G : glycerol solutions, respectively.

変動にもとずく現象と考えられるが，蔭干後藻体を等張液にもどすことによつて放出が促進されることも，脱水にともなう滲透圧の変化がおよぼす作用であると考えられる．藻体を高張液にうつした場合も脱水が考えられるので，各 2.0 溶液の高張液中に 20 分ひたしたものを，等張の各 1.0 溶液にいろいろ組合せをかえてもどし，20 分後に放出の状態をしらべた．その結果は第3図a～fのようである．最初に各 2.0 溶液にひたしたときにあらわれる影響は，実験1の各 2.0 溶液にひたしたときの結果と同じようである．このような 2.0 溶液の作用をうけた各材料を，それぞれ 1.0 等張液にもどしたときの結果は，図にみられるように，放出は基準海水から直接 1.0 各溶液にうつしたときより強い影響があらわれる．このことは高張液にひたすことが乾燥と同じ効果をもたらすことを示している．

5. 外液の濃度と溶質の差が胞子自体ならびに藻体におよぼす影響

基準海水中において果皮をはずし，嚢果中からとりだした果胞子に対する各溶液の影響をみた．処理としては，特定の果胞子を顕微鏡下でマークしておき，カバーグラスの下の海水を濾紙で吸いとると同時に各溶液をスポイドでカバーグラスの下に注入して，胞子自体の直径の増減を2分後に測定した．測定は3～4回行い，その平均値を示したのが第3表である．これによると 0.25 sucrose を作用させた場合 29% の直径の増加を示し，増加率は sucrose，KCl，海水，$CaCl_2$ の順に小さくなる．この傾向は嚢果からの胞子放出促進の傾向とほぼ一致している．低張液のかわりに，2.0 高張液を作用させるとすべて収縮し，$CaCl_2$ では特にいちじるしい．

次にオゴノリ藻体に対する影響を，その表皮層の剝片（長さ1～1.5mm）と輪切りにし

Table 3. Elongation or shrinkage ratio (%) of spore diameter in hypo-or hypertonic solutions (2 min. after immersion).

To hypotonic solution	Elongation ratio	To hypertonic solution	Shrinkage ratio
1.0 Sea Water→0.25 Sea Water	13.1	1.0 Sea Water→2.0 Sea Water	—14.2
1.0 Sea Water→0.25 KCl	20.9	1.0 Sea Water→2.0 KCl	—13.6
1.0 Sea Water→0.25 $CaCl_2$	10.0	1.0 Sea Water→2.0 $CaCl_2$	—17.8
1.0 Sea Water→0.25 Sucrose	29.0	1.0 Sea Water→2.0 Sucrose	—14.7
1.0 Sea Water→Dist. Water	22.2		

Table 4. Elongation ratio (%) of *Gracilaria verrucosa* thallus by immersion to hypotonic solution (5 min. after immersion).

	Strip of cortex	Diameter of thallus
1.0 Sea Water→Dist. Water	3.0	3.7
1.0 Sea Water→0.25 Sea Water	2.3	1.9
1.0 Sea Water→0.25 KCl	2.7	3.0
1.0 Sea Water→0.25 $CaCl_2$	2.0	1.6
1.0 Sea Water→0.25 Sucrose	2.5	2.8

た厚さ 500 μ 内外の枝（直径1～1.5 mm）についてしらべた．胞子の場合と同様に，カバーグラスの下で溶液をとりかえてその伸長をしらべた．処理してから5分後の測定を3～5回行い，その平均値を求めた（第4表）．両方ともその伸長におよぼす各溶質の作用の傾向は胞子の場合と同様であるが，伸長率は胞子の場合にくらべて非常に小さい．

6. 溶質の差が裸出した胞子集団（仁）におよぼす影響

今までの実験における実験は，藻体から突出した嚢果の果口から果胞子が放出される現象（第4図）をみていたのであるが，次に第5－a図にみられるような構造の嚢果の果

Fig. 4. Liberation of carpospores. × ca 10.

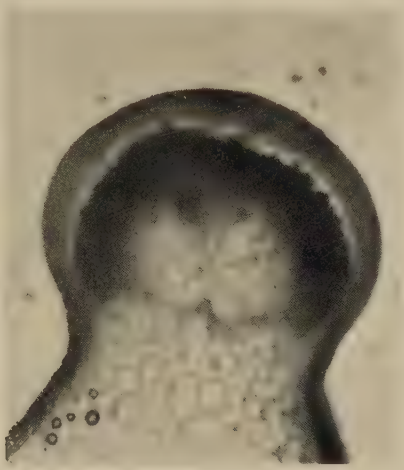

Fig. 5–a. Normal structure of cystocarp. × ca 30.

Fig. 5–b. Denuded nucleus. × ca 30.

Fig. 5–c. Liberation of carpospore from denuded nucleus. × ca 30.

皮をはずして胎座と共にとりだした果胞子の集団に対する影響をみた．とりだした仁は基準海水中では，周囲から熟した 2～3 個の果胞子がはなれるだけである（第5－b図）．この仁の周囲の海水を，実験5と同様にカバーグラス下で各種溶液といれかえて検鏡した．その結果，量的表現は困難であるが，海水を等張の sucrose, KCl, glycerol 溶液にかえたときには，数秒後から続々と仁からはなれてくる果胞子が観察された（第5－c図）この傾向は低張溶液では更に促進される．しかしやはり，$CaCl_2$ 中では抑制される傾向がある．低張液中で游離するときの状態は，果胞子列の前端の果胞子が西洋梨型にふくれ，次に尾をひきながらはなれ，すぐに丸くなる．

7. 実験放出胞子の健全性

いろいろな処理で放出された胞子は，当然その影響をうけるものと考えられる．事実海水の場合でも，低張液にひたすことによつて放出した胞子は膨潤し，数分後に原形質吐出するものがある．そのさいより低張な液中ほど吐出を起す胞子の割合が多く，また起す時間も早い．低張の影響の他に，glycerol, KCl, sucrose, NaCl 等では更に傷害がはなはだしいようである．$CaCl_2$ の場合は抑制されるようである．これに反して，一旦高張液にひたしてから等張液にもどした場合は，放出胞子はすべて健全であつた．高張液の処理が約 20 分の場合，どの溶液で処理された場合も放出胞子には膨潤や吐出がみられず，健全であつた．勿論蔭干処理の場合も同様である．

V. 要　　結

オゴノリ果胞子の放出において，蔭干処理による放出促進現象に関する限り，上記各実験の結果からみられるように，一定条件下のミクロな観察では，藻体あるいは胞子の滲透圧および透過性に関する現象が主な放出促進の要因と考えられる．すなわち実験的には，すべての場合藻体を急に濃度のうすい方にうつしたときに放出が促進されるのは，あきらかに滲透圧の差によつて胞子の体積が増し，膨圧が増加することによると考えられる．実験5でみたように，果皮や柔組織の膨脹率は小さく，胞子のそれは非常に大きい事実は嚢果の構造と考えあわせてこのことをうらずける．すべて測つた伸長率は一次元であるから，体積の膨脹に換算した場合嚢果中の果胞子が低張液中で膨脹する割合は，果皮や柔組織の膨脹率よりはなはだしく大になるために，嚢果中に内圧が生じて果胞子の放出を促進する傾向が生ずるものと考えられる．蔭干による処理によつて放出が促進されるのは，乾燥による脱水のために滲透圧の高まつた胞子がもとの海水にもどされて吸水し，脱水以前より膨脹するためと思われる．同じことが基準海水から一たん高張液にひたした後，もとの海水にもどしたときにおこるいちじるしい放出促進についてもいえる．すなわち滲透圧に落差が生じ，相対的に外液の方が低張になつた場合には放出が促進されるものと考えられる．

溶質のちがいによつて，胞子放出促進に差があることは，これら溶質の透過性の差が胞子の膨脹に関係があるためと思われる．すなわち sucrose, glycerol, KCl 等は透過性が高く胞子を膨脹させやすいと考えられる．胞子放出において，$CaCl_2$ と KCl, $CaCl_2$ と NaCl, $CaCl_2$ と sucrose 各溶液間に拮抗現象がみられることも，イオンあるいは非電解質の透過がこれら溶質が示す独自の放出促進に関係していることを示す．

嚢果の果皮をはずした果胞子の集団に対する各溶質の作用をみると，等張の場合でも，sucrose, KCl, glycerol 等は胞子の游離を促進する．この場合は，はじめから胞子が開放された空間にあるために，膨脹による内圧の増加よりも，果胞子間の連絡物質あるいは異物の膨潤によってはなれやすくなる事の方が游離の原因として妥当と考えられる．

上述の考察をまとめてみると，乾燥あるいは外液の稀釈等によって生ずる滲透圧の落差による放出の促進は，実験的には外液の溶質の透過性もいちじるしく作用するが，自然海水中においては，外液の滲透圧の変化による果胞子の膨脹と果皮などの藻体の膨脹の差から生ずる嚢果内の圧力が果胞子を果口から押し出す第一の原動力になるものと想像される．

海水以外の電解質・非電解質の作用がすべての実験結果において殆ど同じ傾向を示すこと——たとえば sucrose や KCl などのように透過性が高く，又膨潤作用が強いと考えられる溶液中では放出が促進されるということから考えても，ほぼこの仮定が正しいことが想像される．

実験的な急激な滲透圧の変化が，一定条件下のミクロな観察では胞子放出を促進することはたしかであるが，複雑な自然の条件下の紅藻胞子の放出がすべて上述の機作で説明できるとはいえない．たとえばテングサのように日週期のはっきりしたものの日週期的放出の機構は今の所説明できない．オゴノリのように蔭干処理が有効であるものでは，この機作による放出の存在が充分考えられる．少くとも放出の重要な要因の一つであろう．

終にのぞみ，この第2報の研究にいろいろ便宜を与えて下さつた内田教授，大阪市大・三木教授ならびに実験にあたつて有益な御助言・示唆を頂いた大阪市大・高田助教授にあつく御礼申し上げる．

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Summary

The mechanism of the carpospore liberation under different osmotic conditions was studied.

Results of experiments are as follows:

1. A decrease in the osmotic concentration of the external solutions increases the ratio of spore shed cystocarp (Fig. 1). Only when cystocarps are bathed into hypotonic solution of calcium chloride, the effect of the acceleration is scarecely detectable.
2. The spore liberation from cystocarps occurs when the thallus is immersed into natural sea water after the drying in shaded place. But this ratio of spore shed cystocarp increases more effective in such a solution isotonic with natural sea water as glycerol, potassium chloride and sucrose than in natural sea water (Table 2). The marked antagonism is also observed between pairs like $CaCl_2$ and KCl, $CaCl_2$ and NaCl, $CaCl_2$ and sucrose (Fig. 2).
3. By the transfer to solutions isotonic with sea water after dipping in hypertonic ones, spore shed cystocarps increase in number (Fig. 3).
4. Spore diameter increases in hypotonic solutions, especially of potassium chloride, sucrose and glycerol (Table 4).
5. Algal bodies elongate also in hypotonic solutions, but the ratio is not so much as the case of spores (Table 4).
6. Spore liberation from the denuded spore assembly (nucleus) is accelerated even in isotonic solutions of sucrose, KCl, glycerol etc.

From these results, it is concluded that the change of the osmotic concentration of external solutions has a significant effect of inducing carpospore liberation in *Gracilaria verrucosa*. It seems, therefore, that expansion of spores results in an increment of inner pressure sufficient to cause the liberation from carpostome.

有節サンゴモの解剖分類学的研究（追報）

サビモドキ（新属新種）の構造と生殖

瀬　川　宗　吉

Sokichi SEGAWA: Systematic Anatomy of the Articulated Corallines (Supplementary Report)
The Structure and Reproduction of *Yamadaia melobesioides* SEGAWA

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有節サンゴモの解剖分類学的研究（追報）

サビモドキ（新属新種）の構造と生殖*

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1955 年 4 月 12 日受付

筆者は 1940 年から 1949 年[1]に渉り，上記の題目の下に有節サンゴモ各群の代表者を選んで，その解剖分類学的研究の結果を報告した。

その後，筆者は更に現追報の原稿を用意したのであるが印刷事情不良のため現在まで発表を延していた。ここに取扱う有節サンゴモは 1934 年伊豆須崎の海岸に於て採集した極めて特異な種類である（第 1 図）。その外形は Melobesieae 即ち無節サンゴモに極めて類似したものであつて，無節サンゴモを特に専攻する筆者は当時多数の他の無

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*** **Yamadaia** SEGAWA, gen. nov.

Frons crustaeformis. Pars erecta absens. Conceptacula prominentia, per crustam sparsa. Genicula unizonalia, inter crustam et conceptacula praesentia.

Type of the genus: ***Yamadaia melobesioides*** SEGAWA sp. nov.

Characteres iidem ac generis.

Species type: tetrasporic, "Susaki, Izu Prov., Mar. 27, 1939"; female, "Susaki, Izu Prov., Apr. 1941"; male, "Susaki, Izu Prov., Mar. 27, 1939" (in the Herbarium of Mitsui Institute of Marine Biology).

Fig. 1. Matereals. (×4/5)
A. ♂ individual. "Susaki, Izu Prov., Mar. 27, 1939"
B. ♀ individual. "Susaki, Izu Prov., Apr. 1941"
C. ⊕ individual. "Susaki, Izu Prov., Mar, 27. 1939"
D. ♂ individual. "Susaki, Izu Prov., Apr. 1941"

節サンゴモと共に採集し固定し研究を始めた。体の大部分が無節サンゴモ同様皮殻を形成し基質に着生して居るに拘わらず，その生殖窠はその皮殻の中に形成されず必ず外部に超出し，然も皮殻部との境は必ず可動的の膝節によつて区別せられる。発見当時経験の浅かつた筆者はこの特異なサンゴモを分類上如何なる位置に置くべきか甚だ迷つたのである。然しその後無節サンゴモの研究も進み且又有節サンゴモの解剖分類学的研究の進むに従い，次第にその点が明らかになつて来た。加うるに発見当時採集出来なかつた♂個体はまず1939年，♀個体は1941年漸く採集出来，ここに3種の個体も揃い愈々その位置も明らかとなつて来た。そこで此のサンゴモの為新属を設定し，且解剖的の観察を行い，他の有節サンゴモと比較し，その系統に就て論じようと思う。属名 *Yamadaia* は本研究の為終始懇篤なる御指導を下され，校閲の労を執られた恩師山田幸男教授に献じたものである。

あり，和名サビモドキはその外形が余りにも無節サンゴモの某種に類似せるに基いたものである。

体の構造

サビモドキは他の有節サンゴモと異り，体節・膝節交互する直立部を全然有せず，甚しく発達した皮殻部を有するものである。もとより他の有節サンゴモも胞子発生の初め皮殻部をまず作りその上に直立部を作るのであるが，皮殻部の発達は直立部に比して著しく悪く，その為眼に触れないのが普通である。従つて或種の如きは皮殻部の発見に困難を感ずる如き場合もあるのである。本種の皮殻部の放射断面を作つて見るに大体 Melobesieae のものに於ける如く Hypothallium, Perithallium 及び Epidermis の3層を区別する事が出来る。Hypothallium は円柱状の細胞から成り，その細胞糸は体の下部に於いて水平に走り漸次彎曲し直立して Perithallium となる。体の最下部に於いては細胞糸が少しく下方に彎曲するのが普通である。Perithallium の細胞は Hypothallium に於けるよりも短

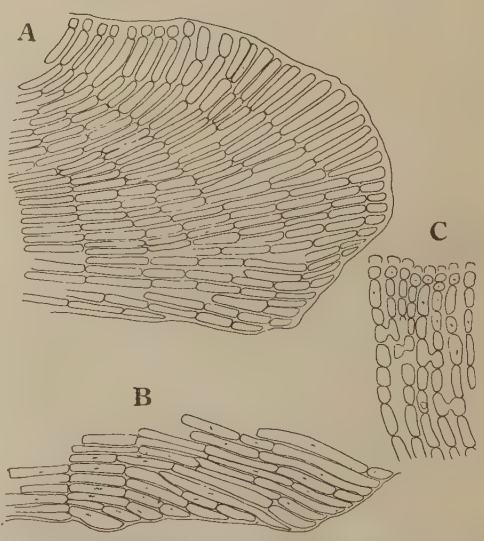

Fig. 2. Longitudinal section of the crust. (×250)
A. Margin.
B. Hypothallium.
C. Perithallium.

く幅も狭い。是等両層に於ける細胞の横の配列は整然たるものではなく相当乱れて居る。然し細胞糸が互に交錯する事は見られない。表皮は断面に於いて正方形に近い細胞の1層から成り，体の古い部分は時に2層以上の事がある。Perithallium の最上層の細胞に冠せられる。然し生長縁には表皮細胞は現れない。又 Hypothallium の下部にも現れない。生長縁には内容の多い長形の分裂細胞となつて生殖窠を有する1体節（Segment）を形成するのである。この際の膝節細胞の核の動向はあまりよく追究されなかつたが，概して云えば膝節細胞の伸長し初めにはずつと下位に存在し，次いで体節細胞の1層（以後の分裂層となる）を分離した当時は核の位置が極めて不同である。次に膝節が完成すれば核の形は長形となり中央部より上位に位置するに到る。

Fig. 3. Development of the node. (×250)

の1群が占めている。

膝節（第3図） 膝節は生殖窠の基部に限つて形成される事は前述の通りであつて，生殖窠は皮殻部の表面に膝節の柄を持つて超出して居る。甚だ長い細胞の1層から成り，両端が Extragenicular portion となつて居る事は他の大多数の有節サンゴモの場合と同様である。

膝節の発生はまづ皮殻部の Perithallium の最上部に相当する細胞の1群が分裂組織に変わる事に初まる。或る一定の長さに達すればその各々の先端には1個の短細胞が分裂される。この時期には未だ Extragenicular portion の分化が認められない。この分裂された細胞がその後の分裂細胞尙膝節の周囲は後に幾分消失し，その膝節細胞によつて形成せられた体節の皮層の部分は皮殻部と直接の連絡を絶たれる。

相異る細胞列間の横の連絡 Hypothallium, Perithallium 及生殖窠を有する体節の髄層皮層を過じ，連絡溝を随所に見出す事が出来る。第二次連絡点は見出すことは出来なかつた。この事は第二次連絡点を有するヒメイソキリ，ヒメカニノテ，*Lithothrix* 等を含む群とは類縁の薄い一つの証拠と思われる。尙前述の分裂層の表面に表皮細胞を持つていない事も又同様一つの論拠となる。

生 殖 窠（第 4, 5, 6 図）

生殖窠は体の上面に散在し，膝節に依つて皮殻部と境する。云わば 1 生殖窠そのものが 1 体節を形成して居る。尙附言する事は sterile の体節と云うものはこの種類に於いては今まで観察されなかつた事である。何れの生殖窠に於いてもその形成の初期に将来窠底となるべき中央部の成長の停止がおこり，その縁部が成長を続けて窠蓋が形成されて行き，窠腔の中央直上に開口を残して完成する。

サビモドキの生殖窠は側生であろうか，頂生であろうか，又はその何れに近いものであろうか。サビモドキの 1 体節より成る直立部は他の有節サンゴモに於ける直立部が最も退化した場合と見做す事が出来る。即ち中間の sterile な体節は発達せずに生殖窠の発達する体節のみ残存したものである。従つて膝節の上に分裂して出来た頂端分裂層の相継ぐ分裂に依つて形成された生殖窠は頂生と云わるべきであろう。解剖的に見て窠底の細胞即ち生殖部位の細胞が髄より起る事からもこの事は云われ得ると思う。

雌性器官（第 4 図） 完成した生殖窠は頂端が鈍円で長倒卵形である。始め成長の停止した窪の中には色素で内容の濃染する原始細胞が並列して見られる。窠底が窠蓋に依つて覆われるに到れば各々の細胞は 1 組の Procarp をつくる。Procarp は 1 個の基部細胞に対して 1 個の Car ogonial branch と 1 又は 2 個の中性細胞が組となつて居る。受精後窠底に癒合細胞が出来る。癒合細胞は薄くて造胞糸が発達する頃になつても尙断面に於いて処々切れて居る場合が多い。造胞糸は癒合細胞の縁部から発達して来る。完成した雌窠の窠腔はもとの空隙よりも拡張の結果広くなり，形は卵形に近い。

Fig. 4. Female conceptacle.
A. Young conceptacle. (×117) B. Procarps. (×167) C. Conceptacle with procarps. (×117) D. Conceptacle with gonimoblast filament. (×117)

雄性器官（第 5 図） 完成した雄性器官を有する体節は他のものに比し，その先端が尖つて居て，外観は紡錘形を呈する。窠腔の形状は断面に於いて半円形で弧の部分が窠底にあたる。窠溝は甚だ長い。窠底は屢々 2 つの急彎曲を認める事が出来。

Fig. 5. Male conceptacle. (×117)
A. Young conceptacle. B. Mature conceptacle.

その彎曲に依つて窠底，窠壁とを区別する事が出来る。精子の母細胞は窠腔の彎曲部一帯に生ずる。即ち窠底と認められる部のみならず窠壁の部にも生ずる。精子の母細胞は密に相並びその各々は長形な未熟精子細胞を着生して居る。精子は長尾状附属物を有するらしく思われるが此の点は明瞭に出来なかつた。

四分胞子嚢窠（第 6 図）

成熟した胞子嚢窠を有する体節は雌性のものと似て居てその外貌は長倒卵形に近い，窠腔は円形で割合に長い窠溝を持つて居る。窠腔は初め小形であるが後に拡張を行い大形となる。将来窠底となるべく成長を停止した組の中には初め分裂細胞と同様内容の豊富な一群の細胞を認める事が出来る。又成熟した生殖窠の窠底には並立した四分胞子嚢が認められる。そしてこれら両者の中間の段階にあるものには往々 Paraphyses を認める事が出来る。

要　　結

以上の結果を要約すれば，

(1) 本種の皮殻部は甚だしく発達し，此に反して直立部は単にその表面に散在せる生殖窠を有する 1 体節に依つて代表せられる。

(2) 皮殻部は Hypothallium, Perithallium 及 Epidermis の 3 部から成る。

(3) 皮殻部の周辺は長形の分裂細胞より成り表皮細胞によつて覆われて居ない。

(4) 膝部は皮殻部と生殖窠の間にのみ形成せられ，1 層細胞より成る。

(5) 隣接細胞列に属する細胞相互の連絡は連絡溝を以つてする。

(6) 膝節細胞相互の連絡は形態的には認められない。

(7) 各種の生殖窠は唯一つの体節に作られ頂生の型に属する。

(8) 各種生殖窠の窠蓋は窠底周囲の部の特別の成長に由来す。

Fig. 6. Tetrasporangial conceptacle. (×117)
A. Young conceptacle. B. Mature conceptacle.

(9) 雌性の生殖窠は長卵形，窠腔の断面は卵形である。

(10) Procarp は 1 の基部細胞に対し，1 の Carpogonial branch と 1 又は 2 の中性細胞より成るのが普通である。

(11) 造胞糸は薄い癒合細胞の周辺からだけ生ずる。

(12) 雄性生殖窠は先端尖り，長紡錘形，窠腔は半円形，窠溝は長い。

(13) 精子母細胞は窠底のみならず窠壁にも生ずる。

(14) 四分胞子嚢窠は長倒卵形，窠腔の断面は円形である。

(15) 四分胞子嚢は窠底のみに生じ，Paraphyses は僅かに生ずる。

本種が狭義の *Amphiroa* 並に *Lithothrix* とはその類縁が遙に遠い事は論の無い処である。又 *Metagoniolithon* も遙かに遠いと思われる。*Bossea, Joculator* と段々類縁が近くはなるがまだまだ共通点は少い。*Calliarthron, Arthrocardia, Duthiea, Cheilosporum* も少しく遠い感がする。結局 *Corallina* か *Jania* に近い事がわかる。此処に注意すべきは Boergesen (1915)[2] が西印度から報ずる処の *Jania pumila* であつて同氏の観察した *Turbinaria* の体上に生育して居た同植物は屢々皮殻部の上に 1 体節を生じ，そのものが生殖窠を有するものである。"The erect filaments are of variable size and development. Some of them consist of a single joint which on its top bears a conceptacle, others are longer, a few times dichotomously divided, ending in conceptacles, if they are not throughout vegetative." と氏は記述している。此の植物を考慮に入れる時はサビモドキ必ずしも独り飛び離れて系統上の位置を取るものとは思われない。*Jania* と或聯関を有する事が予想出来る。然し乍ら 1 体節以上に直立部が発達せぬ事及びこの体節は例外なくその先端に生殖窠を着ける事は 1 独立属としての性質として充分であると認めるのである。尚此処に注意すべきは，この植物の解剖的性質が *Jania* よりむしろ *Corallina* に近いと考えられる節のある事である。もし *Corallina* と *Jania* と相分離すべきものであるとすればむしろ *Corallina* に近く置かるべきものであろう。もし

又 *Corallina* と *Jania* がある学者が考えた如く非常に相近い群であるとすれば勿論是等3者が1つの群となり他の群よりも高位に置かるべきものであろう。

Summary

Yamadaia, a new genus proposed in the present paper, is a very curious articulated coralline. It was discovered by the writer on the coast of Izu Province.

The erect part of the species is reduced to one joint only and the crust is so vigorously developed that, at first sight, it is mistaken for a non-articulated coralline.

The node is unizonal and formed only between the crust and the joint. Thus, the erect part is represented only by a single joint bearing a conceptacle. The conceptacle-bearing joints are scattered on the surface of the crust.

As in crustaceous Corallinaceae, the crust is made up of three layers: the epidermis, the perithallium and the hypothallium. The marginal meristem of the crust is composed of one layer of the long oblong cells and is not covered with an epidermal layer. Only transverse canals are found between cells belonging to different filaments and no morphological connexions are found among cells of the node. The conceptacle has an openning on its top and belongs to the terminal type. The roof of the conceptacle is built up of a special growth of the tissue around the bottom of the young one. The female conceptacle is long obovate in external form, and its cavity is ovate in section. The procarp is composed of one basal cell, one carpogonial branch and one or two sterile cells. The gonimoblast filaments are produced only from the margin of the thin fusion cell. The male conceptacle is long spindle-shaped in outline, and the beak is so long that the conceptacular canal is very long. The spermatangial mother cells are produced not only from the bottom but also from the lateral wall of the cavity. The tetrasporangial conceptacle is long obovate as in the female one, and the cavity is circular in section. The sporangia issue only from the bottom, and are surrounded by paraphyses few in number, in their young stage.

The above mentioned respects show that the present genus has a close relation to *Jania* on the one hand, and to *Corallina* on the other hand. The anatomical characters rather suggest an intimate relation to *Corallina*.

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シダモク（?）に関する観察及びその胚発生[1)]

沢　田　武　男

Some observations on *Sargassum filicinum* Harvey (?) and its embryo-development

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褐藻ホンダワラ科のアカモク ***Sargassum horneri*** Ag. は，我が国に於て極く普通に見られるところの外形に特徴ある植物で，古くから発生学的，細胞学的研究が行われてきたものである．その結果，アカモクは他の種に類のない独特な性質を二・三持つていることが明かとなり，ホンダワラ属中この植物は非常に特異な種類であることが知られていた(猪野 1930, '47; 田原 1940; 須藤 1948).

筆者は先に，従来稀産といわれ且不明な点の多かつたシダモク ***Sargassum filicinum*** Harvey を入手し，主として伊豆の材料について観察を行つたところ，外観はアカモクに酷似しているにも不拘，性質は甚だしく異なつたものであることを報告した(沢田 1955). 即ちアカモクがはつきりした雌雄異株であるのに対し，シダモクは同株で，雌雄両生殖窠は同一生殖器托上に生じ，且場所を違えて存在することである．

しかるに最近，外形はシダモクに非常によく似ているが，気胞がシダモクの様に球形ではなく，楕円形——紡錘形であるモクを天草に於て入手することが出来た．このものについて観察を行つたところ，シダモクに近い性質を持つているが，尚，生殖窠及び生殖器托に関してシダモクには見られなかつた性質もあることがわかつた．また，放出卵を得てその胚発生の初期の経過も観察することが出来たので，これらの結果につき，アカモク，シダモクと比較して述べてみたい．

ただ入手した材料は充分な量とはいえず，採集場所も亦限られた一地方にしか過ぎない．したがつて，以下に述べるシダモクとの相異点が，果して両者の個有の性質に基くものであるか，或いは互に変化しあう同一植物であるか，この点に関する解決は後日に譲ることとし，ここではシダモク(?)として報告する．尚今回取扱つた材料の腊葉標本は，九大農学部水産学教室の Herbarium に保管した．

本文に入るに先立ち，懇篤なる指導と校閲を賜つた九大・農・水産学教室・瀬川宗吉助教授並びに常々激励と御便宜を下されている内田恵太郎教授に衷心より御礼申し上げる．また本植物の採集並びに培養実験を行うに当つて種々御便宜をいただいた九大・天草臨海実験所・塚原博助教授及び塩川司農学士に謝意を表する．

材料とその外形

材料は1955年4月に，中旬と下旬の2回入手することが出来た．何れも天草の通詞島周

1) 日本植物学会九州支部第5回大会(昭和30. 5. 22, 於熊本)で一部を発表した．

辺で漁を行つている底刺網（この地方ではカシアミといつている）にかかつたものであるが，この他にも富岡附近の海岸に於て若干の打揚げ材料を得ることが出来た．

外形は，気胞を除けばアカモク，シダモクと非常によく似ている（Fig. 1）．即ち根はこれらと同様に仮盤状根で，単条の茎がそれより立つている．刺状突起も可成り見られたが，若い体の根に近い部分にはより多いように思われた．基部の缺けた不完全な材料に於て，その長さが1m半以上あるものも得られた．気胞の冠葉や生殖器托，葉等は，夫々アカモク，シダモクのそれらと外観は非常によく似ている．気胞は概して楕円形であるが，中には殆ど球形に近いものや紡錘形のものもあつた．

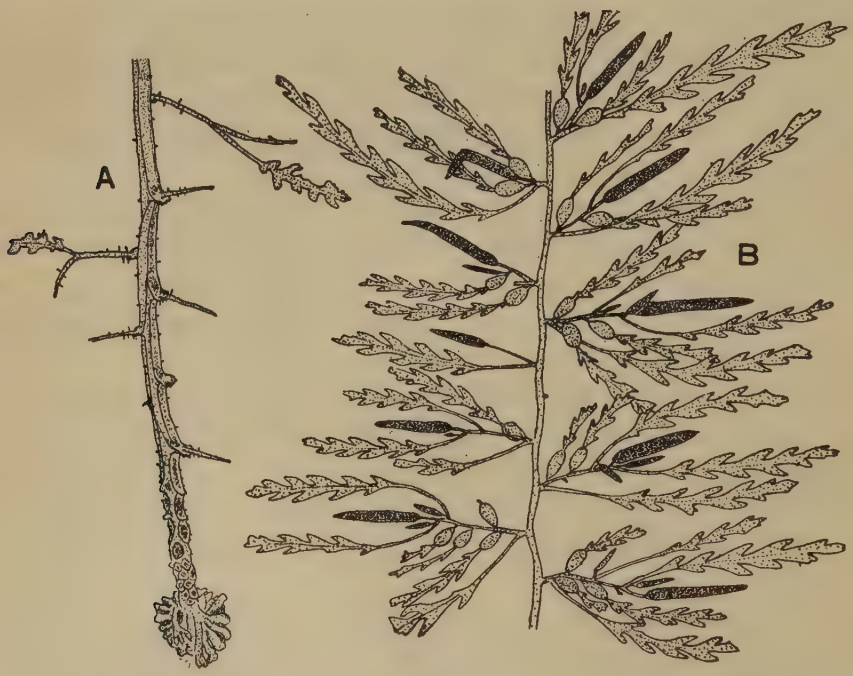


Fig. 1. Basal part (A) and a branch (B) of *Sargassum filicinum* (?) (×2/3).

筆者は先に，シダモクに関して報告した際，その成熟期の項で，3月末に採集した肥後の標本が全く未熟で，未だ生殖器托を付けていなかつたことを述べた．そのものは材料が僅少であつたが，気胞を球形と見做すよりも概して楕円形——紡錘形と見る方が適当と思われる．したがつて，気胞の外形に於て球形と楕円形とで幾分性質に差異があるように思われる現在，シダモク観察の材料とした肥後の標本は，むしろ今度得た本植物と同じものと見る方が妥当であるように考える．[1)]

1）伊豆の標本に於ても極めて少数ではあるが楕円形の気胞が認められた．尚，岡村（1923, '36）はアカモクにも楕円状又は紡錘状の気胞を有するものがあることを述べているが，このものの生殖器托に関しては記述がない．従つてそれがアカモクであるか或いはシダモク（？）であるかについては，その材料に於ける生殖器托の検討がなされるべきであると思う．

生　殖　器　托

アカモクの成熟期は可成り早いものの一つとして考えられているが，天草では4月下旬頃，卵の放出状態から推察して最盛期のように思われた．この植物でもアカモクと全く同様な状態のものが同時期に見られたが，同時にまた，生殖器托を未だ有していない個体も若干得られたので，この種の成熟期は大体アカモクと同じか，或いは多少晩いのではないかと思われる．

生殖器托は大体長さ 2～3cm，太さ 2～3mm の円柱状で，外観はアカモクの雌，またシダモクのものと殆ど異なるところはない．しかしこれらや，我が国に於て普通に産する多くのホンダワラ類と甚だしく異なる点は，生殖器托の内容即ち生殖窠の性質である．この植物に於ては，ハハキモクや *Eusargassum* 亜属のタヲヤメモクその他でよくあるように，雌雄両生殖窠は生殖器托の各部に混在していて androgynous である．しかも尚，雌雄2種の生殖窠の他にも，系統上，下位のものでよく見られるところの hermaphrodite の，即ち雌雄同窠の生殖窠（Fig. 2）も少数ながら存在することは興味深く思われる．Hermaphrodite の生殖窠は，生殖窠としては原始的なものと考えられており，この点ホンダワラ属のように高位の群に於てもこのような生殖窠が存在することは，外国では少数例知られているが（Grunow 1915），本邦のものではまだその報告をきかない．

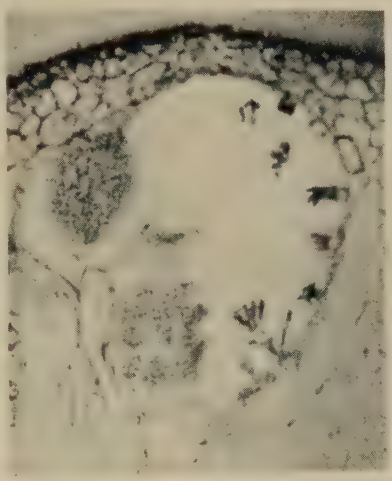


Fig. 2. Hermaphrodite conceptacle in *Sargassum filicinum* (?) (×130).

Hermaphrodite の生殖窠に於ては，雌雄はその窠中に場所を選ばず互に混生するのが殆どのようであるが，*Bifurcaria tuberculata* Stackhouse や *Cystoseira barbata* J. Agardh その他では，窠中で雌雄は夫々別々に存在しているものもあるようである．即ち窠口に近い部分の半分には雄，その奥の方半分は雌と観察されている（Sauvageau 1912, etc.）．本植物の hermaphrodite の生殖窠では，窠中で夫々両側に位置を占めているものが多かつた．何れか一方が他方を圧し，少部分に限定してしまつている場合も見受けられたが，雌雄が混在している場合は今のところ未だ観察されていない．

シダモクに於て雄の生殖窠は，生殖器托の基部附近からだけ検出されたが，この植物に於ては雌・雄並びに hermaphrodite の3種の生殖窠は，生殖器托のどの部分からも検出することが出来た．そこで生殖器托内に於ける3種の生殖窠の分布状態を，連続切片を作つて調べたところ，大体雄は下位，就中生殖器托の基部1割位の部分にはより多く，雌はそれ以外の殆どの部分を占めていた．このことは可成りシダモクに似た傾向を持つているようである．尚 hermaphrodite のものは非常に僅少であるが，幾分基部の方に多いように思われた．

Androgynous の生殖器托内に於ける雌雄両生殖窠の存在状態については，Setchell (1935, '36, '37)，山田 (1942)，猪野 (1943) その他の報告がある．即ち同一種であつても株によつて，生殖器托中に含まれる雌雄両生殖窠の割合が異なつている．例えばある株では雄生殖窠が，またある株では雌生殖窠が優勢で，外形も夫々雄性または雌性を示し，雌雄生殖窠の同数宛ある株では外形も中間的であるという．今回の植物がこのような所謂 "dioico-androgynous" の性質を持つものではないかと考えたが，どの株の任意の生殖器托に於ても，雄生殖窠の数が雌のものより多い場合は見られなかつた．勿論，入手した材料は充分な量ではないので，直ちにそのような性質の無いことを断定は出来ぬとしても，少くとも筆者の入手したものに於てはその可能性を肯定させるようなものは見られなかつた．尚生殖器托については，個体差の他にも地域的変化，時期による雌雄何れかの先熟等も考慮して，今後もシダモクや本植物の蒐集に努め，差異や疑問の点に関して追究を行いたい．

卵及び胚発生

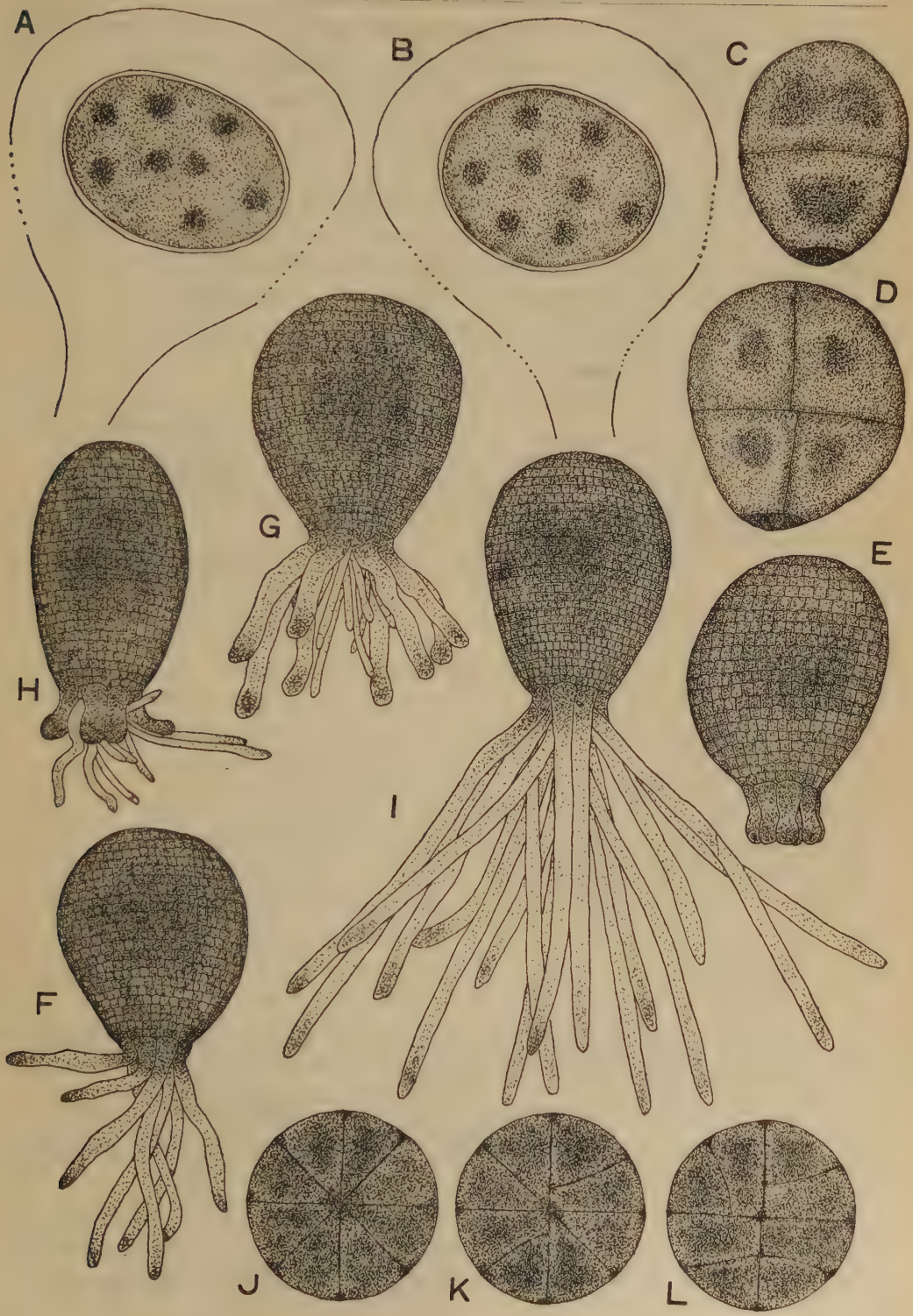
方法　この実験に用いた材料は，1955年4月28日（旧暦3月7日）の早朝，漁を終えて二江に帰つて来た底刺網にかかつていたものである．午前6時30分頃，網が引揚げられてから半乾状態のままであつた材料を胴乱に納め，自転車で約1時間の距離を富岡の九大・臨海実験所まで運んだ．持ち帰つてから直ちに海水を満たしたエナメル・バットに入れ，水道栓から海水を流して暫時材料の検討を行つた．その後，放出卵の附著した生殖器托を硝子容器の静水中に移して培養を続けた．培養海水の温度は 18～20°C で，毎日換水を行い，仮根数を確認出来る段階に発育するまで観察を続けた．

観察　材料を採取した時には既に卵は放出されており，膠質状物質に包まれて生殖器托表に附著していた．しかし実験室に持ち帰つて直ちに検鏡したところ，8 箇の核が夫々褐色の色素体に囲まれて散在しているのが認められたこと，及びその後の受精卵の発育経過から推察して，材料を採取した時は卵の放出後あまり時間が経つていなかつたと考えられる．

卵の放出は他のホンダワラ類と同じく，週期的に，各生殖器托に於て基部から上部に向い，帯状に2～3回位に分けて行はれるものと思われるが，今回観察したものの中には，生殖器托を基部から 3/5～4/5 位，放出卵が取巻いているものも多かつた．

精子の卵への侵入位置は，須藤 (1948) が数種のホンダワラ類で明かにした結果，アカモクでは蔵卵器中膜に帯状の膨潤部があることを報告した．他の種類には見られないこの性質は，最初国枝・須藤 (1940) によつてアカモクに於て知られていたものであるが，シダモクでも同様であることは筆者が先に報告した．今回の植物に於てもこれらと同じく精子の侵入位置として帯状の膨潤部があるのを観察することが出来た (Fig. 3, A)．稀れではあるが卵からはずれた下の位置にも別に1つ，合計2つ持つているものも1例見られた (Fig. 3, B)．

Fig. 3. Discharged oogonia and young embryos of *Sargassum filicinum* (?). A, discharged oogonium in 8-nucleate stage. B, the same with 2 girdle-like dissolved portions (abnormal). C-I, successive stages in embryo-development; I, young embryo, 7–8 days after oogonium discharged. J-L, segmentation in rhizoid cell; J, normal; K, L, abnormal. A-I (×130), J-L (×330).


A
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放出卵は，球形に近いものもあるが主に楕円形か所謂卵形であり，その大きさは大体長軸 265μ，短軸 205μ 位で，アカモクやシダモクのものに非常に近い大きさである．

受精卵の発育は，アカモクで見られるのと大差ない様式で行はれる．初め放出卵で見られた8核の中，7核が消失して先ず第1分割壁が卵の中央を横断して走り，上下の2細胞に分ける．次に第2の分割壁が下部の細胞で同方向に走り，仮根細胞を生ずる．この仮根細胞は大きさが直径 85μ 位で，アカモクのものと略一致している．この仮根細胞は，極面から見て中心で直交する2つの細胞分割壁により4細胞に分割されるが，この次の分割壁もまたそれぞれ中心を走るので，結局仮根細胞は放射状に並んだ8細胞に分けられる（Fig. 3, J）．しかし少数例ではあるが放射状に並ばない異常例も見られた（Fig. 3, K, L）．

仮根細胞が放射状に分割される例としては，従来アカモクだけで知られていたのであるが，この植物に於ても同様の分割を行うことがわかつた．

この8箇の細胞は夫々伸長を始めるが，多くの場合幼胚の長さ乃至その半分位まで伸びた頃，その内側からも別な仮根群が伸び始めて来る．このものは外側のものに較べると最初は明かに細いけれども，急速に伸長し，また太さを増して程なく両者の差別はつかないようになる．そして卵放出後数日と思われる頃には，内外の合計10数本から20本前後の仮根が見られるようになる．

田原（1913）はアカモクの仮根の伸長に関し，8箇に分かれた仮根細胞の各々が伸び出しきらないうちに，仮根細胞に起因しないところの仮根の一群が前者の内側から伸び始め，前者よりも先に長く伸びることを報告している．しかし筆者が福岡市外津屋崎・九大水産実験所附近で6月上旬採取したアカモクの幼胚を培養した結果では，必ずしもそのようなものばかりとは限らなかつた．即ち多くのものは前述の如く外側のものが可成り伸びてから内側に細い仮根が現れ，程なく前者に追いつくものであつた．且仮根数も 20 本を越えるものが多数観察された．勿論天草のシダモク（？）でも津屋崎のアカモクでも，従来の報告通り内部の仮根群の方が先に伸びた例も若干は見られた（Fig. 3, H）．従つてこの植物がとる胚発生の様式は，アカモクと同じ型と考えても差支えないと思う．

尚，シダモクについては未だ観察する機会を得ていないが，今回の結果からも考えて，先にシダモクに関する報告で予想した通り，これらと同じ型の発生を行うのではないかと思う．

摘　　要

1．筆者はシダモクによく似た植物を天草に於て入手し，観察を行つた結果，生殖器官並びに胚発生に関して若干の知見を得ることが出来た．

2．外形はアカモク，シダモクと非常によく似ているが，気胞が大概楕円形である点，これらとは少し異なる．が，その他では仮盤状の根，刺状突起のある単条の茎，葉，冠葉等，外観は殆ど異なるところはない．

3．成熟期は，アカモクと略々同時期か或いは多少遅れるのではないかと思われる．

4．生殖器托の外形は，アカモクの雌またシダモクのものと殆ど同じであるが，生殖窠の性状に関しては大いに異なつた点がある．即ち雌雄の両生殖窠の他に，hermaphrodite

の生殖窠も存在することは興味深く思われるが，尙これら3種の生殖窠は，1生殖器托内に於てどの部分からも見出された．又 hermaphrodite の生殖窠にあつては，雌雄はその窠内に於て互に混生することなく，窠口をはさんで両側に位置するものしか見られなかつた．雄生殖窠が生殖器托の基部の少部分に特に多く存在することは，シダモクに非常によく似た性質である．

5．1生殖器托に於て，卵の放出は基部から上部に向つて2～3回位に分けて行うものと思われる．

6．放出卵は，大きさが略々 265×205 μ の楕円形で，アカモク，シダモクのものと大体一致し，膠質状物質に包まれて暫く生殖器托表に附著している．精子の侵入位置としてはアカモク，シダモクと同様に，蔵卵器中膜に帯状の膨潤部が観察された．

7．胚発生の経過は，アカモクと同様な型をとると考えられる．即ち仮根細胞は放射状に8箇に分割され，その後，仮根細胞に起因しない仮根群が内側から伸び始めて，合計10数本から20本前後の仮根が伸長を行う．

8．仮根細胞に起因する仮根群が或る程度伸びた後に，その内側から別な仮根群が現れる例の多かつた事実は，従来アカモクについて報告されていたものと必ずしも一致しない．しかし，アカモクに於ても今回の植物と同様な例を福岡市外の材料で観察することが出来たので，胚発生に関してはアカモクに同じと考えても差支えないと思う．

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Résumé

In the previous paper the author reported some interesting characters of the sexual organs in *Sargassum filicinum*. Notwithstanding the strong resemblance between *S. horneri* and *S. filicinum* in the external shape excepting the difference in the vesicular shape remarked by W. H. Harvey, they are distinguishable in sexuality: the former is strictly dioecious while the latter monoecious and androgynous in the receptacle.

In April 1955, he happened to obtain another alga with the ellipsoidal or somewhat ovato-fusiform vesicles. It may not be identified with a typical form of *S. filicinum* and also not undoubtedly with *S. horneri*. In this paper the author intends to report the results of observations on this alga especially on the sexual organs and the embryo-development, and also to try some comparison with those of *S. horneri* and *S. filicinum*.

The material were collected with some bottom gill-nets, chiefly at Hutae, Amakusa Islands, Higo Province. The results are summarized as follows:

1) The alga in question bears strong resemblance to both *S. horneri* and *S. filicinum* in having the disco-fibrous root, the fine spinous processes on stem, the cylindrical receptacles, and the alternato-pinnatisected leaves, but is different by having the ellipsoidal vesicles (Fig. 1).

2) The alga seems to mature about in April to May in Amakusa Islands, so is it in *S. horneri*, but comparing with the latter the former may be later slightly.

3) The receptacles are androgynous; besides male and female conceptacles the same receptacle harbours hermaphrodite ones (Fig. 2), but the number of hermaphrodite conceptacles is indeed less than the others in each receptacle. The male conceptacles are not more than the female's, and they are found in any part of the receptacle, not being confined in the basal part as in *S. filicinum*; though they gather more in the basal part. In the hermaphrodite conceptacle the oogonia and the antheridial hairs are not intermingled, but occupy the side position to each other.

4) Discharge of oogonia takes place periodically, and the discharged portion advances acropetally in each receptacle, as is usual in many other members of the genus *Sargassum*.

5) Discharged oosphere is ellipsoidal measuring ca $265 \times 205\,\mu$, which is

surrounded by a thick layer of mucilaginous substance. Around the outer wall of the thick layer there is observed a girdle-like dissolved portion as is reported on *S. horneri* (Kunieda & Suto 1940) and *S. filicinum* (Sawada 1955).

6) The embryo-development takes the same course with *S. horneri*. The rhizoid cell is segmented into 8 radially arranged cells ("radial 8-cell type" by Inoh 1930), and yet those 8 rhizoids developed from the rhizoid cell another group of about 7 to 15 rhizoids are observed to begin growth.

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貝類廃棄部の利用に関する研究 —I.
鶏の生長及び産卵に及ぼす効果*

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Studies on Utilization of Wastes in Processing Shell-Fish—I.
Effect on Chick Growth and Egg Production.

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Takeo KOYANAGI, Shogoro UCHIDA, Goro KATO and Sasaku TANAKA

The present paper deals with the effect of boiled extracts of *Venerupis philippinarum* (I), *Pecten laqeatus* (II), *Mactra sachalinensis* (III), and *Anadara inflata* (IV) upon chick growth, egg production, weight of anterior hypophysis and testis, and length of humerus and femur.

All the boiled extracts given at a 3.6% dry basis level showed the same growth promoting effect as the fish meal at 20% level (Table 1). The difference in weight of anterior hypophysis between the chicks fed the boiled extract of (I) or (II) and the control chicks fed only all-vegetable protein ration was significant at 5% level (Table 2). The rate of egg production in one-year old hens showed a marked response to a supplementation of boiled extract of (I) at 3.6% level (Table 3). In case of 5—month old hens, extracts of (I) and (IV) at a 3.6% level were as effective as the fish meal of a 20% level to an earlier start of egg production and to raise the egg production rate (Table 4).

I. 緒 言

わが国には 1952 年の統計によると年産 1,888 万貫に達するアサリが生産されている。食用に供する場合にはなま剝よりゆで剝の方が多いので，その際にえられる煮汁は莫大な量に達する。アサリ煮汁中には生貝全体の約 1.4 % のエキス成分が移行するので，乾物として約 26 万貫の資源が年々廃棄されていることになる。アサリ以外の貝類についても同じ様な未利用部が考えられる。それ故にこれを利用して蛋白栄養資源の確保に資する目的をもつて本研究に着手した。

米国では Fish soluble を養鶏，養豚に利用した場合の効果については最近多数の研究があるが，貝類煮汁についてのこの種の研究はない。依て著者等は先づ福岡県に饒産するアサリ (*Venerupis philippinarum*)，イタヤ貝 (*Pecten laqueatus*)，ウバ貝 (*Mactra sachalinensis*) 及びアカ貝 (*Anadara inflata*) 等の煮汁が鶏の生長及び産卵率にどの程度有効であるかについて検討した。なお従来この種の研究では鶏の生長，産卵率或は孵化率等の外面的観察だけに限つた数値の報告が多いが，本研究では脳下垂体前葉，生殖腺重量，骨長等をも測定することにより APF の作用機構についても探究した。

II. 実 験 方 法

1955 年 4 月 11 日受理
* 本報告の大要は昭和 27 年 4 月東京に於ける日本水産学会大会に発表した。

1. 煮汁の製造及び濃縮： イタヤ貝煮汁： 約 500 *L* の淡水の熱湯にイタヤ貝を順次に加え，煮汁の量が大約 500 *L* を保つように時々湯を添加しつゝ，4,500 *kg*（煮熟貝柱 200 *kg*）を処理した。この煮汁の内 30 *L* を約 70°*C* で減圧濃縮してシラツプ 5.0 *kg*（乾物 3.61 *kg*）を得た。収量は貝全体に対し 1.33 %，貝柱に対して 30 % に当る。

ウバ貝煮汁： 業者よりえた煮汁（比重 1.18 *Be* 22°）30 *L* を濃縮し，シラツプ 8.4 *kg*（乾物 2.22 *kg*）をえた。

アサリ及びアカ貝煮汁： 当業者のゆで剝身製造時に副生した煮汁を減圧濃縮釜を用いて約 70°*C* で濃縮したシラツプである。

なお煮汁製造試験を実験した。熱湯 5 *L* にアサリ 20.4 *kg* 或はアカ貝 19.1 *kg* を徐々に加え処理した結果，貝殻はアサリ 8.9 *kg* ，アカ貝 9.8 *kg*，貝肉はアサリ 3.4 *kg*，アカ貝 3.9 *kg*，煮汁はアサリ 8.88 *L*，アカ貝 7.43 *L* をそれぞれ得た。この煮汁を約 70°*C* で減圧濃縮してアサリシラツプ 538 *g*（乾物 297 *g*）及びアカ貝シラツプ 435 *g*（乾物 222 *g*）を得た。この結果から煮汁に移行する固形分はアサリの場合，貝全体に対し 1.46 %，アカ貝の場合 1.16 % であり，乾燥剝身に対しては，アサリでは 42.9 %，アカ貝では 32.7 % の多量に達している。各煮汁濃縮物の化学的組成を Table 1 及び 3 の脚註に示した。

2. 育雛試験： 供試鶏は 1952 年 8 月 16 日に孵化した白色レグホン種の ♂ 雛である。1 区は 13 羽として，室内においた金網底をもつバツタリーに収容した。9 月 24 日より 11 月 19 日まで飼養管理し，毎週給餌前に体重を測定した。試験区には Table 1 の脚註に示した基本飼料 A に乾物として 3.6 % の試料を添加したものを与えた。対照区には試験区の粗蛋白量に近似させるために基本飼料 A に 3.5 % の大豆粕を添加した基本区と魚粉 20 % を含む基本飼料 B を与えた標準区とを設けた。いづれの区にも風乾飼料の 30～60 % の大根葉或いは龍舌菜を混和して給与した。給餌量は常に基本区と同一にし paired feeding の方式をとつた。なお試験終了後，試験雛を屠殺し，脳下垂体前葉及び睾丸の重量，上腕及び大腿骨の長さを計測した。

3. 産卵試験： この試験は次に示すように 2 年鶏及び 1 年鶏について行つた。試験 (1) では 1950 年春孵化した白色レグホン種の内で 1 年間の産卵検査の結果，200～220 個を示したもののみを選び，5 羽を 1 区として，1951 年 12 月 4 日より 1952 年 3 月 20 日まで飼養管理した。なお供試鶏は実験開始迄は Table 3 の脚註に示した魚粉を含む基本飼料 C で飼育した。

試験 (2) では 1951 年 8 月 23 日に孵化した白色レグホン種の ♀ 雛を魚粉を含まない基本飼料 D 及び緑餌で 3 ケ月間飼育した後，発育度の平均した健康雛を選び，8 羽を 1 区として，1952 年 1 月 22 日より 3 月 20 日まで飼養管理した。

いづれの試験に於ても試験鶏は戸外の三階造りのバッタリーに収容し，試験区には基本飼料 D に乾物として 3.6 % のアサリ及びアカ貝の煮汁を添加したものを与えた。他方試験区の蛋白量に近似させるため大豆粕を 3.5 % 増補した基本区と魚粉を含む基本飼料 E を与えた標準区とを設けた。なお給餌量は 1 日 1 羽当り大約 120 *g* とした。

Trap nest の準備がなかつたので，各個体の産卵数は測定できなかつたが，1 区の総産卵数と 2 月 7 日より 3 月 20 日までの卵重量を測定した。また試験 (2) では産卵開始期をも記録した。

III. 結 果 及 び 考 察

A. 鶏雛の生長に及ぼす効果

各区の平均体重増加は Table 1 に示す通りで，8 週間の飼育結果では基本区に対しウバ貝区，標準区，イタヤ貝区及びアサリ区の順に差を生じた。即ち之等の試料はいづれも雛の生長促進に有効であり，しかも試験区の蛋白量は魚粉 20 % 添加した標準区の蛋白量より約 2 % 少かつたに拘らず標準区と近似の効果を示した。特に注目される点は試験区では基本区と同一量の飼料を摂つたにも関らず，より大きな増重を示したことであつて，煮汁の有効成分は飼料効率即ち飼料の体内に於ける利用率を向上させたことがわかる。

なお，試験終了後，試験雛を屠殺し，脳下垂体前葉及び睾丸の重量，上腕及び大腿骨の長さを計測した。その結果は Table 2 に示した通りで，睾丸重量は変異が極めて大で比較し難く，また骨長も差が認められ

なかつた。然し脳下垂体前葉の重量はアサリ区及び標準区は基本区に対し 5% の危険率で有意の差が認められた。ウバ貝区及びイタヤ貝区は生長が優れていたにも拘らず，脳下垂体前葉重量は基本区と有意の差が認められなかつた。この点については更に検討する必要がある。

Table 1. Growth response of male chicken to boiled extracts.

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Basal diet		Average gain in body wt. (gm)					Gain % of control
			Kinds[4]	Crude protein %	Initial	2 wk	4 wk	6 wk	8 wk	
12	Ubagai[1]	3.7	A	17.9	190	167	281	513	747	133
11	Itayagai[2]	3.6	A	17.2	167	143	243	448	678	121
12	Asari[3]	3.6	A	17.5	178	168	296	420	642	115
10	None[5]	—	A	18.2	173	159	218	425	560	100
7	Fish meal	20.0	B	19.6	135	176	314	415	707	126

1 *Mactra sachalinensis* : moisture 25.7%, crude protein (dry basis) 20.7%, salt (dry basis) 55.8%. 2) *Pecten laqueatus* : moisture 27.8%, crude protein (dry basis) 23.0%, salt (dry basis) 50.7%. 3) *Venerupis philippinarum* : moisture 28.7%, crude protein (dry basis) 33.1%, salt (dry basis) 44.0%. 4) Basal diet A used was composed of corn 50, wheat embryo 20, wheat bran 10, rice bran 5, soybean meal 15, NaCl 0.5, $CaCO_3$ 3. Basal diet B : corn 50, wheat embryo 15, wheat bran 10, rice bran 5, fish meal 20, NaCl 0.5, $CaCO_3$ 3. Both diets were supplemented radish- and *Lactica indica*-leaves at 30~60 per cent level. 5) To equalize the protein-content, 3.5 per cent level of soybean were added.

Table 2. Response in several organs of chicks fed the boiled extracts.

	Ubagai	Asari	Itayagai	None	Fish meal
No. of chicks	12	12	10	10	7
Anterior hypophysis (g)	7.0±0.9*	8.0±1.2	7.6±1.3	6.7±1.1	8.1±0.75
"t" test+		2.6	1.6		2.9
Testis (g)	307±93	388±64	409±124	423±316	563±304
Humerus (cm)	7.4±0.3	7.2±0.6	7.1±0.3	7.1±0.5	7.1±0.5
Femur (cm)	8.0±0.3	7.7±0.5	7.6±0.4	7.6±0.5	7.6±0.6

* Standard error + n=15, ε=0.05, t_0=2.131. n=20, ε=0.05, t_0=2.086

Table 3. Response in weight and production of eggs from two-year old hen fed the boiled extracts.

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Basal diet		Egg production[4]		Egg wt. (g)[5]	
			Kinds[3]	Crude protein %	Total number	%	Total	%
5	Asari	3.6	D	20.2	251	125	5360	109
5	Akagai[1]	3.6	D	20.6	201	100	5314	108
5	None[2]	—	D	20.9	200	100	4909	100
5	Fish meal	20.0	E	20.6	173	86	4014	82

1 *Anadara inflata* : moisture 35.6%, crude protein (dry basis) 42.8%, salt (dry basis) 29.8%. 2) To equalize the protein-content, 3.5 per cent level of soybean were added. 3) Basal diet C used was composed of corn 50, wheat bran 15, rice bran 15, fish meal 20, NaCl 0.5, $CaCO_3$ 3. Basal diet D was composed of corn 40, wheat bran 20, rice bran 20, soybean meal 20, NaCl 0.5, $CaCO_3$ 3. Basal diet E was composed of corn 40, wheat bran 20, rice bran 20, fish meal 20, NaCl 0.5, $CaCO_3$ 3. All the birds were fed the basal diet C before the experiment. All the diets were supplemented with the leaves shown in Table 1. 4) Dec. 4 '51~Mar. 20 '52 5) Feb. 7 '52~Mar. 20 '52

Table 4. Response in weight, number and date of starting egg production from one-year old hens fed the boiled extracts.

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Basal diet		Egg production[2]		Egg wt.[3] (g)		Date of starting egg production
			Kinds	Crude protein %	Total number	%	Total	%	
8	Asari	3.6	D	20.2	138	114	5852	121	Feb. 7
8	Akagai	3.6	D	20.6	185	153	7343	151	Feb. 3
8	None[1]	—	D	20.9	121	100	4843	100	Feb. 6
8	Fish meal	20.0	E	20.6	194	160	6955	143	Jan. 22

1) To equalize the protein-content, 3.5 per cent level of soybean were added. 2) Jan. 22. '52～Mar. 20 3) Feb. 7. '52～Mar. 20

All the birds were fed the basal diet D for 3 months before the experiment.

B. 産卵率に及ぼす効果

（1） 二年鶏の産卵率向上効果： 産卵数及び卵重量を計量した結果は Table 3 に示す通りで，アサリ区は食塩の過多のために試験期間中軟便を続けていたに関らず，産卵率は高く，卵重量は大であつた。アカ貝区の産卵数は基本区と差が認められなかつたが，後期の卵重量は著るしく大で基本区を凌駕した。魚粉を含む標準飼料区は予期に反して全く効果が認められなかつたが，試験2で著るしき産卵率向上効果を示したのは使用魚粉の APF 含量が異つていたためか或は試験鶏の能力が不揃であつたためかと思われる。

（2） 一年鶏の産卵開始期並に産卵率に及ぼす効果： 産卵数及び卵重量を計量した結果及び産卵開始期は Table 4 に示す通りで，アサリ区の産卵数及び卵重量は基本区を凌駕し，明らかに産卵率向上効果を示したが，産卵開始期は基本区と同じであつた。しかしアカ貝区は基本区より僅かに早く産卵し始め，産卵数及び卵重量は基本区のそれを遙かに凌駕した。他方魚粉を含む標準区は基本区より2週間前から産卵し始め，顕著な産卵率向上効果を示した。

なお二つの産卵試験を比較すると，試験（1）の各試験区の効果は試験（2）の各区の効果よりそれぞれ劣つた。この原因については次のような理由があげられる。即ち試験（1）の供試鶏は試験開始前約1年間魚粉を含む普通飼料を与えていたので，体内に多量の APF を蓄えていたと考えられるが，試験2の供試鶏は孵化後直ちに APF 欠乏飼料を与えたため，体内に APF が欠乏していたと考えられる。従つて二つの試験の効果の差は供試鶏の体内 APF の多寡によつて生じたのであろう。

IV. 総　　括

1. アサリ，ウバ貝及びイタヤ貝の煮汁濃縮物は乾物として 3.6％ を植物性の基本飼料に添加することにより鶏雛の生長を著るしく促進した。その効果は魚粉 20 ％ を含む標準飼料の効果と同程度であつた。

2. 育雛試験の雛の脳下垂体前葉重量をみると，アサリ煮汁区と基本区との間には有意の差が認められた。

3. アサリ煮汁濃縮物を基本飼料に乾物として 3.6％ 添加したものは，白色レグホン種の二年鶏の産卵率を著るしく高めた。

4. アカ貝煮汁濃縮物を基本飼料に乾物として 3.6％ 添加したものを一年鶏に5ケ月間与えると，魚粉を含む標準飼料と同様に産卵開始期が早くなり，また産卵率は向上した。アサリ煮汁 3.6％ 添加区も多少劣るが産卵率を向上した。

終りに本研究は昭和 26 年度の科学試験研究費により実施したものである。試料の調製及び飼養管理に尽力された菅原憲典，奥山俊司，土井良平次郎，行徳志信，松尾新一，佐伯種敏の諸氏に深謝の意を表する。

貝類廃棄部の利用に関する研究—Ⅱ.
鶏の生長及び産卵率に及ぼすアサリ煮汁の効果*

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Studies on Utilization of Wastes in Processing Shell-Fish——Ⅱ. The Effect of the boiled Extract of *Venerupis philippinarum* upon Chick Growth and Increase of Egg Production during the Molting Season.

Tetuo TOMIYAMA, Yasuo YONE, Yoshitaro KATO, Masao OKAMOTO, Makoto YAMAOKA, Takeo KOYANAGI, Shogoro UCHIDA, Yukio MURAKAMI and Sasaku TANAKA

A study has been made of the effect of the boiled extract of *Venerupis philippinarum* upon 6-month growth of chicks *fed ad libitum* and upon egg production during the molting season.

In growth assay were used female Single comb white Leghorn chicks hatched from eggs obtained from hens fed a normal diet. As shown in Fig. 1 the boiled extract given at 2~3% dry basis levels for 6 months showed a marked effect on the growth. The hens which had been fed practical ration containing fish meal were used for the egg production test. It has clearly been shown that the addition of the boiled extract at a 3.4 % dry basis level prevented a reduction in the egg production during the molting season and was able to cut the consumption of fish meal at least 10 per cent (Fig. 2 & Tabble 3).

Ⅰ 緒 言

著者等[1]は既にアサリ，アカ貝，イタヤ貝及びウバ貝の煮汁が鶏の生長促進，産卵率向上，産卵開始期の短縮等に有効なることを報告した。しかしその生長試験では paired feeding の方式をとり，しかも試験期間は比較的短期間であつた。またその産卵試験は最も産卵率の高い 12 月より 3 月迄の期間に行つた。従つて長期に亘つての自由摂餌による育雛試験の実施が望ましく，また産卵率の低下する換羽期の産卵に及ぼす効果を知ることは産業的に重要である。更にまた養鶏用の所謂完全飼料には一般に 15~20% の魚粉を必要とするが，現在の魚粉の価格ではかように多量を使用することは経営上困難であるから，他の適当な APF の給源を用うることによつて魚粉の使用量を低減できれば，経営上極めて有利となるに違いない。

依て著者等は上記のような見地から本研究を行つた。その結果によるとアサリ煮汁の添加は初生雛より 7 ケ月の飼育に於て著明な生長促進効果を示し，また換羽期の産卵率低下を防止し，魚粉の節約のみでなく，魚粉の価値をも高めることが明かとなつた。

Ⅱ 実 験 方 法

1. アサリ煮汁濃縮物

当業者の製造したアサリ煮汁を有明海水産試験場で減圧濃縮したものである。その化学的組成は水分31.5%，乾物中の粗蛋白質 34.9%，食塩 29.6% であつた。

1955 年 4 月 11 日受理 * 本研究は昭 27 和年度文部省科学試験研究費で支弁し，その大要は昭和 28 年 4 月東京に於ける日本水産学会大会に発表した。

2. 育雛試験

供試雛は 1952 年6月 17 日に孵化した白色レグホン種の♀で，60 羽から体重の揃った 20 羽を選び，10 羽を1区とし，6月 23 日より9月 30 日迄飼育し，10月1日に各試験区の平均体重に近い雛を5羽づゝ選び飼育を経続した。なお6月 23 日より9月3日までは室内においた金網底をもつバッタリーに収容し，9月4日以降は戸外の金網底或は竹格子底をもつバッタリーで飼育した。

試験区には 1952 年6月 23 日より9月 30 日迄は基本飼料に乾物として3%のアサリ煮汁濃縮物を添加し，10月1日以後は乾物として2%を添加した。なお基本区には試験区と蛋白量を近似させるため大豆粕 3%を添加した。基本飼料は6月 23 日より9月 30 日までは Table 1 に示した基本飼料Aを用いた。但し9月4日より9月 30 日までは葉酸，ビタミン K_3 及びレシチンの投与を中止し，基本飼料の 50% に相当する大根葉を与えた。10月1日以降は基本飼料Bを用い，緑餌としては大根葉，龍舌菜を与え，自由摂取とした。

Table 1. Composition of basal diet.

Basal diet	Corn	Soybean meal	Rice bran	Whea embryo	Wheat bran	$CaCO_3$	Manganized salt	Olive oil	Crude protein (%)
A*	51	25	10	10	—	3	0.5	1	18.0
B+	47	30	10	—	10	3	—	—	21.7

* Folic acid 4mg, 2-methyl-naphtoquinon 1mg, lecithine 10g and V.A 20,000 I.U, per Kg diet, were supplemented. Basal deit B was supplemented radish- and *Lactica indica*-leaves at a 50 per cent level.

3. 産卵試験

試験鶏は 1952 年2月 11 日及び 23 日に孵化した白色レグホンで，試験開始前2ケ月間は一般の成鶏用飼料で戸外に飼育したものゝ内，体重 1,300～2,000g の健康なもの 20 羽を選び，1試験区に5羽を用いた。底部を竹格子にした戸外のバッタリーに収容し，1952年7月8日より試験飼料の投与を開始した。7月 12 日には各区とも約半数が産卵を開始したので産卵数の測定を始め，10月 31 日迄経続した。なお各個体の産卵能力は不明であるから，Trap nest を用いて各個体の産卵数を求め，駄鶏は産卵率の計算から除外した。

試験区としては魚粉 20% 含有の標準区，魚粉を 10% としてアサリ煮汁5%添加した区，魚粉を5%としてアサリ煮汁5%添加した区及び大豆粕 24% 含有の APF を含まない区を設けた。

アサリ煮汁濃縮物は適量の水にとかし試験配合飼料中に均一に混和して与え，投餌量は各区共1日1羽当り 120g とした。なお各試験区の蛋白含量を近似させるためにアサリ煮汁5%一魚粉5%区のみに大豆粕を5%添加した。また各試験区共大根葉及び龍舌菜を飼料の 17% 相当量与えた。各試験区の飼料配合率及び蛋白含量の詳細は Table 2 に示した。

Table 2. Compositon of diet used.

Groups of expt.	Fish meal	Corn	Wheat bran	Soybean meal	Salt	Boiled extr.	Crude protein (%)
Fish meal (F.M)	20	40	40	—	0.5	—	19.1
Extr. 5%+F.M 10%	10	45	40	—	—	5	15.9
Extr. 5%+F.M 5%	5	45	40	5	—	5	15.9
Soybean meal	—	40	40	24	0.5	—	21.5

All the diets were supplemented radish- and *Lactica indica*-leaves at a 17 per cent level.

Ⅲ 結果及び考察

A. 生長に及ぼす効果

供試鶏5羽の内，各区共それぞれ1羽ずゝ試験期間中に死亡したので，4羽の平均生長曲線を Fig. 1 に示した。アサリ煮汁区の生長は1週より基本区の生長を凌駕し，飼育開始後35日後（7月 28 日）の増加体重比率は基本の 100 に対し 129，114 日（10 月 15 日）後は 135 であり，その生長促進効果は顕著であつた。なお生長の極限に近づいた 224 日後(1953 年2月1日)

は 114 で，なお体重差が認められた。

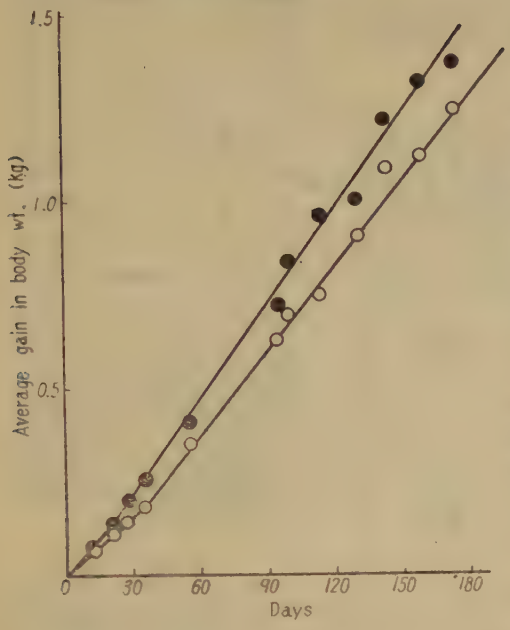

Fig. 1 Growth response of chicks to boiled extract of *Venerupis philippinarum* at 2~3% level.
● extract of *Venerupis philippinarum*.
○ no supplement.

先ず産卵中止日数について比較すると，アサリ5%—魚粉 10% 区が 43 日で最も小さく，アサリ5%—魚粉5%区が 93 日，魚粉 20% 区が 141 日，大豆粕 24% 区が 184 日であつて，アサリ煮汁の効果が顕著であつた。

全試験期間中の産卵率はアサリ5%—魚粉 10% 区が最高で，アサリ5%—魚粉5%区がこれに次ぎ，魚粉 20% 区及び大豆粕 24% 区は遙かに劣り，第1位のものの 81% に相当する。換羽期間，即ち試験開始後8週より 14 週までの産卵率をみると，試験区の優劣は更に著るしく，アサリ5%—魚粉 10% 区及びアサリ5%—魚粉5%区が最も優り，魚粉 20% 区は蛋白含有量が前2者に比し多少多いにも拘らず産卵率は遙かに劣り，また蛋白含量の最も多い大豆粕 24% 区は極めて低値を，第1位のものの 36% にしか達しなかつた。こゝに注目される点はアサリ煮汁を添加した区ではいづれも換羽期間の産卵率は全期間の産卵率と大差がなかつたのに対し，魚粉区及び大豆粕区では換羽期間の産卵率は全期間の産卵率より著しく低下し，アサリ5%—魚粉 10% 区の産卵率に比し約36~50%にしか達しなかつた。

アサリ煮汁濃縮物の B_{12} 含量を Euglena 法で測定した結果，乾物 100g 中 234 μg で基本飼料 100g に 7.02 g の B_{12} を与えたことになる。他方アサリ煮汁の APF を活性炭で分別した結果（次報参照）によると，B_{12} 以外に未知の生長因子の存在が知られたので，アサリ煮汁添加による生長促進はこれ等因子の綜合的作用によるものと推察される。

B. 換羽期の産卵率低下防止

産卵試験の結果は Table 3 及び Fig 2 に纒めた通りである。産卵効果を時期的に解析するために，10日間以上産卵を中止した日数（産卵中止日数と仮りに呼ぶ），全試験期間中の産卵率及び全般に換羽が観察された期間中（9月6日より 10月17日迄）の産卵率を算出した。

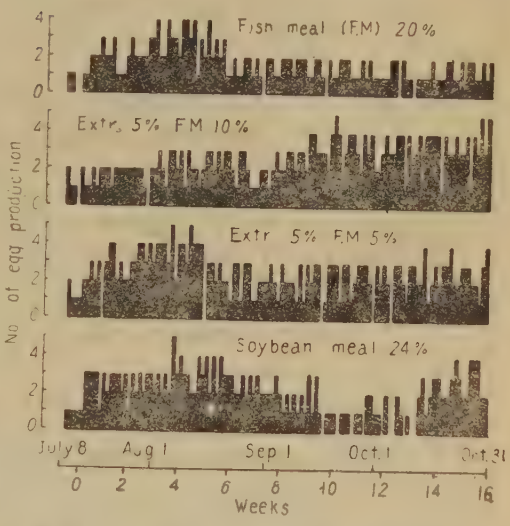

Fig. 2 Change in egg production during molting season.

またこの換羽期間中の産卵率低下の程度は換羽の程度と並行することが飼養中観察された。かようにアサリ煮汁濃縮物を飼料の5%添加するのみで，少くとも魚粉 10% の節約ができるだけでなく産卵中止日数は短縮され，特に換羽期の産卵率低下が防止される点は経営上重要視されねばならない。たゞこゝに疑問に思われる点は，魚粉のように APF を含むと思われるものが換羽期の産卵率低下を防止する効果の低かつた事実である。これは恐らくは魚粉の有効成分の大部分が魚粉製造中煮汁及び圧搾汁中に失われるので，原料の

Table 3. Biweekly change of egg production in each experimental group.

Supplements	No. of chicks	Biweekly production of egg							Total number (%)	8~14wk egg producton number(%)	Date of start of egg production	Days of no egg production
		0~2	2~4	4~6	6~8	8~10	10~12	12~14	14~16				
Fish meal 20%	923	10	10	9	8	8	5	4	11	195 (43.5)	55 (32.7)	VII/ 9	0
	924	10	9	7	0	0	2	1	0			VII/ 8	73
	926	0	10	9	11	11	12	11	10			VII/26	0
	977	6	9	10	1	1	0	0	0			VII/14	68
Extr. 5% Fish meal 10%	2079	1	2	1	0	3	6	11	11	292 (52.1)	136 (61.7)	VII/18	33
	907	13	11	13	7	11	13	12	19			VII/ 8	0
	1530	0	4	10	11	11	5	2	3			VIII/1	21
	909	10	11	6	8	13	9	10	11			VII/ 8	0
	1586	0	0	0	0	6	11	13	11			IX /10	0
Extr. 5% Fish meal 5%	918	10	12	4	0	0	0	8	10	283 (50.5)	93 (44.2)	VII/ 9	53
	919	10	8	7	10	10	9	9	10			VII/ 9	0
	920	8	8	7	1	1	1	0	1			VII/13	69
	921	0	11	12	12	11	12	8	10			VII/28	0
	922	5	11	10	7	8	10	6	6			VII/20	0
Soybean meal 24%	939	11	8	1	0	0	0	0	7	236 (42.1)	49 (23.3)	VII/11	75
	940	12	13	12	8	0	2	5	12			VII/ 9	24
	941	11	12	11	10	6	0	1	0			VIII/14	46
	942	0	2	8	3	6	9	5	12			VIII/7	12
	943	0	2	11	11	8	1	6	10			VII/ 6	27

The renewal of feather was observed to take place in each group from 8- to 14-weeks, and the degree of the renewal ran parallel with decrease in egg production.

種類，饌度，煮熟方法，圧搾程度等によって魚粉中の有効成分量が一定しないことに原因するであろう。かように考えると煮熟及び圧搾の充分な従来良質の魚粉とされていたものが却って前記のように有効性を示さないことが理解される。従って最近ヨーロッパでは煮汁及び圧搾汁を魚粉に混合した Whole fish meal が販売され始めた。かような見地から見ると，この有効成分を豊富に含むアサリ煮汁は魚粉の節約のみでなく魚粉価値を高める意味からも高く評価されるべきであろう。

Ⅳ 総　括

1. アサリ煮汁を乾物として2～3%添加した飼料を初生雛に6ケ月間給与すると，著明な生長促進効果がみられた。

2. アサリ煮汁濃縮物の B_{12} 含量を Euglena 法で測定した結果，乾物 100g 中に 234 μgであつた。

3. 1952年2月孵化の白色レグホン種を産卵開始直前の7月初めより 10 月末迄 16 週間飼養して，産卵率の変動を比較検討した結果，アサリ煮汁濃縮物を添加した場合には換羽期の産卵率低下が防止された。また試料5%（乾物として 3.4%）の添加により少くとも 10% 程度の魚粉の節約ができるようである。

文　献

(1) 富山哲夫外七名：日水誌 21, 838 (1955)

貝類廃棄部の利用に関する研究—Ⅲ. アサリ煮汁の APF の分別*

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Studies on Utilization of Wastes in Processing Shell-Fish—Ⅲ. Fractionation of growth factors in extract of *Venerupis philippinarum*.

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A fractionation by treating with activated charcoal has been made of growth factors for chicks which were found in the boiled extract of the clam, *Venerupis philippinarum*.

A filtrate fraction which was obtained on treating a slightly acidic solution of the boiled extract with the adsorbent, still possessed growth factors in a conciderable amount. This is not case with bovine liver extract. An eluate from the adsorbate with a dilute sodium hydroxide solution showed a growth promoting activity nearly equal to that of the filtrate fraction. The assay of vitamin B_{12} by *Euglena gracilis* indicated that 76 per cent of the vitamin in the extract passed into the non-adsorbed fraction, and that only 0.19 per cent was found in the eluate. It follows, accordingly, that growth accelerating activity manifested by the eluate must be due to the presence of some unidentified growth factor or factors, probably vitamin B_{15} postulated by TOMIYAMA and YONE[2].

I. 緒 言

著者等[1]は既にアサリ煮汁が鶏の生長促進並びに産卵率向上に著るしい効果を示すことを確認し，またアサリ煮汁中には乾物 100 g 当り 254 γ の多量の B_{12} が存在することを報告した。他方，富山，米[2]は牛肝中には微酸性で活性炭に吸着され，苛性ソーダで溶出される新らしい生長因子即ちビタミン B_{15} が存在し，牛肝の示す雛生長促進効果はビタミン B_{12} によるものよりもむしろ B_{15} に由来することを明らかにした。

従つて本研究ではアサリ煮汁の示す APF 効果にはビタミン B_{12} 以外の因子も関与しているか否かを検討した。即ち，アサリ煮汁濃縮物を活性炭に吸着される区分と吸着されない区分に分劃し，各区分の示す鶏雛生長促進効果と各区分のビタミン B_{12} 含量との関係につき検討考察した。また本研究では育雛試験終了後に試験雛を屠殺し，脳下垂体前葉，生殖腺，甲状腺重量，骨長等を測定し生長効果の機構を明らかにしようと試みた。

II. 実 験 方 法

1. 試料の調製

1952 年 3～5 月に有明海で採集したアサリ 1,360 kg (7石1斗) を煮熟水 12 L に投入し，水を入れ換

1955 年5 月 30 日受理

* 本研究の大要は昭和 28 年4 月東京に於ける日本水産学会年会に発表した。

えずに煮沸して剝身 205 *kg*，煮汁 241 *L* を得た。更に煮汁を 70°*C* 以下で減圧濃縮し，濃縮物 18.35 *kg* を得た。次いで煮汁濃縮物 2 *kg* に 10 倍量の水を加え，塩酸で pH 4.5 として，硅藻土及び活性炭をそれぞれ 1.4 *kg* づゝ加えて吸着し，吸着物は 10 *L* の水で洗つた後，苛性ソーダ水溶液（pH 9）で吸着物を溶出し，中和後減圧濃縮した。洗滌液は非吸着液と共に中和後減圧濃縮した。各試料の組成は Table 1 に示されている。

2. 育雛試験

この試験は次に示すように 2 回行つた。試験 A では 1952 年 4 月 17 日に孵化した白色レグホン種の ♂ 雛 43 羽を孵化後 33 日目より 4 区に分けて試験を開始した。試験開始前までは電熱保温器をもつ金網底のバツタリーで飼育したが，試験開始後は屋外のバツタリーに収容し，5 月 20 日より 7 月 20 日迄の 62 日間飼養管理した。毎週の体重の測定は給餌前に行つた。試験区には煮汁濃縮物 5 *g*（乾物 3.3 *g*）より得た吸着物 0.58 *g*（乾物 0.2 *g*）及び非吸着物 2.87 *g*（乾物 1.6 *g*）を Table 1 に示した基本飼料 100 *g* に添加し，無添加の基本区と対比した。なおいづれの区にも風乾飼料の 50 % の龍舌菜を混和して与えた。給餌は Paired feeding の方式をとつた。試験雛は飼育終了後に屠殺して脳下垂体前葉，睾丸，甲状腺の重量及び上腕骨，大腿骨の長さを計測した。

試験 B では 1952 年 6 月 17 日に孵化した白色レグホン種の ♀ 雛を用いた。100 羽から体重のそろった健康雛 40 羽を選び，1 区 10 羽として保温室においた金網底をもつバツタリーに収容した。実験は 6 月 23 日より 7 月 21 日に亘り実施し，毎週体重を給餌前に測定した。試験区には煮汁濃縮物 4.5 *g*（乾物 3 *g*）より得た吸着物 0.38 *g*（乾物 0.13 *g*）及び非吸着物 4.5 g（乾物 2.5 *g*）を Table 1 に示した基本飼料 100 *g* に添加し，無添加の基本区と対比した。なお給餌及び給水は自由摂取とした。

III. 結 果 及 び 考 察

A. 中雛育雛試験

育雛試験の結果は Table 1 に示す通りで，煮汁濃縮物の効果が最も大きく，非吸着物，吸着物の順で，第 9 週後の体重増加比率は基本の 100 に対し，煮汁濃縮物区は 152，吸着物区 116.8，非吸着物区 122 であつた。なお各試験区の生長と基本区のそれとの差に対する t 検定の結果では Table 1 に示すようにいづれの区も 5% の危険率で有意の差が認められた。各試験区の飼料摂取量 100 *g* 当りの増加体重量は Table 1 に示す通りで，煮汁区，非吸着物区，吸着物区，基本区の順に少くなり，生長度と概ね一致している点は

Table 1. The growth response of chicks to boiled extract of *Venerupis philippinarum* and to fractions adsorbed and non-adsorbed on activated charcoal. (May 20 '52～July 20)

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Average body weight in gm.				"t" test[4]	Total gain in weight over total feed intake (%)
			Initial	After 3 wks	After 6 wks	After 9 wks	Average gain	
11	Boiled extract[1]	3.3	147.8±4.6*	278.7	429.8	639.7±10.1	491.9±12.2	7.7	17.8
11	Adsorbed fraction[2]	0.2	142.5±2.5	251.3	375.4	503.0±5.8	360.4±13.0	2.4	13.6
11	Non-adsorbed fraction[3]	1.6	136.9±3.8	238.9	375.2	530.4±12.4	393.5±11.0	4.0	14.2
12	None		140.9±3.7	243.5	351.5	463.5±14.1	322.5±12.3		12.4

1) Moisture 33.4 %, crude protein (dry basis) 37.3 %, salt (dry basis) 28.9 %. 2) Moisture 65.6 %, crude protein (dry basis) 21.4 %, salt (dry basis) 37.8 %. 3) Moisture 44.2 %, crude protein (dry basis) 26.4 %, salt (dry basis) 33.3 %. 4) n=21, 0.05……t_0=2.08.

* standard error of the mean. Basal diet used was composed of corn 51, soybean oil meal (expeller) 25, wheat embryo 10, rice bran 10, $CaCO_3$ 3, manganized salt 0.5, liver oil (500 I.U) 1. Basal diet was supplemented *Lactica indica* leaves at a 50 per cent level.

Table 2. Reponse in several organs of chicks fed the boiled extract, adsorbed fraction or non-adsorbed fraction.

	Boiled extr.	Adsorbed fraction	Non-adsorbed fraction	None
Anterior hypophysis (g)	5.7±0.27*	5.6±0.24	5.3±0.24	5.5±0.23
Testis	282±26.8	163±14.1	180±10.5	150±16.2
Thyroid gland (g)	30.5±1.48	28.6±0.93	27.2±0.81	27.6±1.24

* standard error of the mean.

興味が多い。

次に解体し睾丸，脳下垂体前葉，甲状腺重量を測定したが，その結果は Table 2 に示す通りで，脳下垂体前葉重量には殆んど差なく，甲状腺重量も腺自体の性質から見てこの程度の相違は日常の生理的状態で容易に変移することが考えられるから，この数字から大きな意味をくみとることは無理である。睾丸の重量は体重に正比例し，特にアサリ煮汁区は群を抜いて発達した。なお上腕骨及び大腿骨の長さは変異の差が極めて大であつたため比較し難かつたので結果は省略した。

B. 初生雛育雛試験

前項の中雛の試験によるとアサリ煮汁濃縮物は顕著な生長促進効果を示したが，吸着物の効果は非吸着物の効果より小であつた。このことは牛肝中の APF が微酸性で完全に活性炭に吸着されることと一致しないので，アサリ煮汁の APF 中には活性炭に対する吸着性の点で牛肝の APF と異るものがあるのか，或いは APF の吸着を妨害する物質の存在により見かけ上，非吸着物が有効であつたかの二つの原因が考えられる。従つて本試験では初生雛を用い，試験 A の結果を追試すると共に Euglena 法を用いてアサリ煮汁濃縮物中の B_{12} の活性炭に対する行動を追跡した。

Table 3 Growth response of chicks to a boiled extract of *Venerapis philippinarum* and to fractions adsorbed and non-adsorbed on activated charcoal.

(June 23 '52～July 21)

No. of chicks	Supplements*	Amt. of supplements per 100 g diet. Dry dasis	B_{12} per 100 g diet	Average biweekly body wt. in gm				"t" test×
				Initial	After 2 wks	After 4 wks	Average gain	
9	Boiled extract	3.0	7.2 μg	38.8±1.7+	119.1	238.1±5.8	199.5±5.2	6.8
8	Adsorbed fraction	0.13	16.2 mμg	42.0±1.2	113.1	207.4±4.8	165.3±4.9	2.7
9	Non-adsorbed fraction	2.5	9.9 μg	42.0±1.2	112.5	207.4±7.8	165.4±7.3	2.4
9	None	——	——	40.2±1.2	102.8	182.2±6.9	142.0±6.5	

* Composition of supplements and basal diet were shown in Table 1.

\+ n=15, 0.05……t_0=2.131, n=16, 0.05……t_0=2.12.

× standard error of the mean.

育雛試験の結果は Table 3 に示す通りで，飼育開始後 4 週目には基本区の生長に対し，アサリ煮汁濃縮物区が最も優れ，吸着物区及び非吸着物区がこれに次いで生長が大であつた。この試験では吸着物と非吸着物の効果には差が認められなかつた。各試験区の生長と基本区の生長との差の t 検定をした結果でよいずれの区も 5% の危険率で有意の差が認められた。

非吸着物が試験 A の結果と同様に生長促進効果を示した原因を探究するため，Euglena 法を用いて各試料の B_{12} を定量した。その結果は Table 4 に示す通りで，煮汁濃縮物中の B_{12} の 76% は活性炭に吸着されなかつた。他方自己消化した牛肝中の B_{12} は微酸性で活性炭に完全に吸着されるから，アサリ煮汁中には活性炭による B_{12} の吸着を妨害する物質が存在するのであろう。活性炭吸着部を稀アルカリ水溶液で溶出した部分には，牛肝の場合と同様に B_{12} 量は極めて少く，しかも煮汁中のビタミン B_{12} の 0.19% がこの部分に移行したに過ぎなかつた。従つて本実験の条件でえた吸着部の効果は明らかに B_{12} 以外の未知 APF によるものであり，非吸着物の効果は恐らく主として B_{12} の効果と推察される。

Table 4. Content of vitamin B_{12} in the samples used.

	V. B_{12} μg%	Relative yield of fractions %	Distribution of V. B_{12} of the extract into two fractions.	
			μg	%
Boiled extract	160	100	160	100
Adsorbed fraction	4.3	7	0.3	0.19
Non-adsorbed fraction	220	55	121	75.5

IV. 結　　論

1. アサリ煮汁を飼料に乾物として 3.3～3.0 % 添加したものを中雛及び初生雛に 9 週間或いは 4 週間給与すると，著るしい生長促進効果が認められた。

2. アサリ煮汁の APF を微酸性で活性炭処理し，吸着部と非吸着部とに分劃し，吸着部は稀アルカリ水溶液で溶出して供試した。煮汁よりの各部の収量を計算に入れて煮汁乾物 3.3 % 相当量をそれぞれ飼料に添加し生長効果をみた結果，中雛では非吸着部がやゝ優り，初生雛では両者同程度の生長を示した。基本区の生長との差に対する t 検定の結果では 5 % の危険率で有意の差が認められた。

3. アサリ煮汁中の B_{12} はそのうちの 76 % が活性炭に吸着されなかつた。また活性炭吸着・稀アルカリ溶出部には僅かに 0.19 % が移行したにすぎなかつた。従つて活性炭吸着部の生長促進効果は B_{12} 以外の未知 APF によることが明かとなつた。

4. アサリ煮汁，活性炭吸着部及び非吸着部はいずれも煮汁乾物 3.3 % 相当量を中雛に投与すると，飼料効率が増加した。

5. 体内所見の結果，煮汁区及び非吸着物区の睾丸重量は基本区のそれに比し大であつたが，脳下垂体前葉及び甲状腺の重量は本実験の範囲では各区の間に著明な差は認められなかつた。

終りに本研究は昭和 27 年度の文部省科学試験研究費により実施したものである。試料の調製及び飼養管理に尽力された管原憲典，奥山俊司，土井良平次郎，行徳志信の諸氏に深謝の意を表する。

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貝類廃棄部の利用に関する研究—Ⅳ
鶏の生長に及ぼすイタヤ貝内臓液化物の効果*

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Studies on Utilization of Wastes in Processing Shell-Fish.—Ⅳ.
The Effect of Viscera-Extract of *Pecten laqueatus* on Chick Growth

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Takeo KOYANAGI, Shogoro UCHIDA, Goro KATO and Sasaku TANAKA

Preceeding report[1] showed that addition of the boiled extract of several shell-fishes at about 3% level resulted in a noticeable chick growth response. Since it can be presumed that the response may be most probably due to the APF which was extracted from the viscera, a study has been made to see the effectiveness of an autolyzed viscera to chick growth and further to fractionate the growth-promoting principles. The results can be summarized as follows.

1. Supplementation of an autolyzed viscera at a 1.7 per cent dry basis level gave a remarkable growth response of chicks fed a basal ration of all-vegetable protein.

2. Addition to 100 g basal ration of 0.27 g of the activated charcoal adsorbate which is equivalent to 2.8 g of the dried autolyzate, resulted in growth promotion of one-month old chicks during six-week feeding as contrasted to a negative result on addition of 1.35 g of the non-adsorbed fraction.

3. It has been found that the adsorbed fraction promoted both body growth and the development of the gonad, whereas the non-adsobate apparently promoted only the latter.

Ⅰ. 緒 言

有明海及び筑前海にはそれぞれタイラギ及びイタヤ貝が饒産し，これらは貝柱を利用するので，加工に当って副生する内臓は現在肥料に供されているにすぎない。このような貝柱のみを利用している貝類の内臓は全国的にみると相当な量に達するので，これらから蛋白栄養食源を回収するのを目的として本研究を行つた。

筆者等[1]はイタヤ貝煮汁が顕著な鶏雛の生長促進効果を示すことにつき既に報告した。この促進効果は貝の内臓の熱湯抽出物に由来すると推察される。よつて内臓が鶏雛の生長をどの程度促進するかについて検討し，更らに有効成分の分別を試みた。

Ⅱ. 実 験 方 法

1. 試料の調製：イタヤ貝内臓液化物，活性炭吸着物及び非吸着物は次のようにして調製した。1951年4月及び1952年1月にそれぞれ玄海で採取したイタヤ貝をなま剝した後，貝柱を除いた残部に醋酸を加えて実験室に運び，これを富山の液化法[1]で処理し，水溶部を70°C以下で減圧濃縮した。この内臓液化物は細目の笊で濾過した程度であつたから，内臓液化物の有効成分を分別するためには，先ず液化物を水に溶

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* 本報告の大要は昭和27年4月に東京に於ける日本水産学会年会に発表した。

した後，遠心分離して不溶部を分ち，これを多量の水で数回洗つたものを遠心上澄液に合わせて水溶部を調製した。次いで水溶部に活性炭を加え．pH 4.5 で吸着した。活性炭吸着部は稀苛性ソーダ水溶液（pH 9）で溶出した後，更に 65% エタノールを加え煮沸して溶出した。溶出液（活性炭吸着物と略称する）及び活性炭に吸着されない濾液（非吸着物と略称する）はそれぞれ中和後 70°C 以下で減圧濃縮した。

2. 育雛試験：試験 A では 1951 年 5 月 16 日に孵化した白色レグホン種のさ雛を用いた。育雛箱の一部に電熱器をおいた 100 羽用の育雛箱を二分して一区に 30 羽を収容した。なお使用した育雛箱の底部は金網でなかつたので，毎日糞の掃除に留意した。1951 年 5 月 18 日より 6 月 8 日まで飼養管理し，毎週給餌前に体重を測定した。イタヤ貝内臓液化物区には Table 1 の脚註に示した基本飼料 A 100g に乾物として 1.7 g のイタヤ貝内臓液化物を添加し，ビタミン B_{12}+葉酸区には基本飼料 A100g に対し，ビタミン B_{12} 1.5 μg 及び葉酸 200ug を添加した。なお対照区としては基本飼料 A のみを与えた基本区と魚粉 20% を含む飼料 B を与えた標準区とを設けた。なお標準区のみに緑餌として基本飼料の 50% 相当量の龍舌菜を与えた。給餌及び給水は自由摂取とした。

試験 B では 1951 年 12 月 19 日に孵化した白色レグホン種の雛を用いた。約 100 羽を Table 1 の脚註に示した魚粉を含まない基本飼料 A で 1 ケ月間飼育し，この中より体重の揃つた健康雛 60 羽を選んだ。1 区 15 羽として電熱保温した室内に設備した金網底をもつバッタリに収容した。1952 年 1 月 18 日より 2 月 29 日まで飼養管理し，毎週給餌前に体重を測定した。なお試験終了後解体し，脳下垂体前葉及び睾丸重量，上腕骨及び大腿骨の長さを測定した。イタヤ貝内臓液化物区には基本飼料 A 100 g に乾物として 2.8 g のイタヤ貝内臓液化物を添加し，吸着物区及び非吸着物区にはイタヤ貝内臓液化物よりの収量に準拠して乾物として 0.27 g の吸着物，1.35 g の非吸着物をそれぞれ添加した。基本区にはイタヤ貝内臓液化物区の粗蛋白量に近似させるため 3.5% の大豆粕を補添した。なおいづれの区にも緑餌として基本飼料の 25～30 % 相当量の大根葉を与えた。給水及び給餌は自由摂取とした。

Ⅲ. 結果及び考察

A, イタヤ貝内臓液化物及び葉酸・ビタミン B_{12} の効果：

育雛試験は 13 週間行つたが，4 週以後コクシジウムが多発し，多数の試験鶏が斃死したので 3 週迄の平均体重を Table 1 に示した。その結果をみるとイタヤ貝内臓液化物は最も有効で，20 % の魚粉を含む飼料 B を与え，また緑餌も与えた標準区と殆んど同じ効果を示した。内臓液化物区の平均増加体重比率は植物蛋白のみの基本飼料 A を与えた対照区の 100 に対し 133 を示した。葉酸及び B_{12} の併用は生長促進効果を全く示さなかつた。Stockstad 氏等[2] は雛の生長に充分な B_{12} 量は 1.5 μg % であることを明かにしたが，このような異る結果を生じた原因については次のようなことが考えられる。Stockstad 氏等はビタミン B_{12} を

Table 1. The growth response of chicks to extract of viscera of *Pecten laqueatus*. (May 18～June 8, '51)

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Basal diet		Average weekly body wt. in gm.				No. of survival
			Kinds[3]	Crude protein %	Initial	After 1	After 2	After 3	Average gain
30	Extract of viscera[1]	1.7 g	A	17.2	39	46.6	61.6	89.2	50.2	25
30	V. B_{12}[2]+ Folic acid	1.5 μg 200 μg	A	16.4	39	44.8	57.2	77.6	38.6	28
30	None		A	16.4	39	45.0	54.1	76.8	37.8	27
30	None		B	19.6	39	45.0	61.0	87.9	48.9	26

1) Moisture, 42.0% ; on dry basis, crude protein. 44.1%. 2) Vitamin B_{12}, "Fresmin" 1cc= 15 μg B_{12}; Folic acid, "Foliamin" 1cc=15 mg folic acid. 3) Basal diet A used was composed of corn 50, wheat embryo 20, wheat bran 10, rice bran 5, soybean meal 15, NaCl 0.5, $CaCO_2$ 3. Basal diet B : corn 50, wheat embryo 15, wheat bran 10, rice bran 5, fish meal 20, NaCl 0.5, $CaCO_3$ 3. Basal diet B was supplemented with *Lactica indica* leaves at a 50 per cent level.

欠乏させた雛を供成しているのに対し，本研究では一般成熟飼料を与えた種鶏からえられた雛を用いたので，B_{12} が卵を経て供試雛に移行しているために B_{12} の添加に反応しなかったのであろう。

B．内臓液化物中の有効物質の分別

前実験でイタヤ貝内臓液化物は鶏雛の生長を促進することが明らかになったので，本実験では内臓液化物中の APF の分別を試みた。著者等[3]は既にビタミン B_{12} 及び著者等が発見したビタミン B_{15} はいずれも微酸性で活性炭に吸着されるが，B_{15} は苛性ソーダ稀溶液（pH 9）で溶出されるのに対し，B_{12} は苛性ソーダ稀溶液では溶出されないで，煮沸 60% エタノールで溶出されることを明かにした。依て本研究では内臓液化物を活性炭に吸着される区分と吸着されない区分に分別し，吸着物は苛性ソーダ稀溶液（pH 9）と煮沸 60% エタノールで溶出したものを用いた。また前試験に於ては緑餌を与えなかつたためかコクシジュウムに犯されたものが多く，斃死が頻発して試料の有効度を判定するのに困難であつたので，本研究ではいずれの区にも緑餌を与えた。育雛試験の結果は Table 2 に示す通りで，試験開始後 6 週目には吸着物区が最も優

Table 2. The growth response of chicks to extract of viscera of *Pecten laqueatus* and to fractions adsorbed and non-adsorbed on activated charcoal. (Jan. 18～Feb. 29, '52)

No. of chicks	Supplements	Amt. of supplements per 100g diet. Dry basis (g)	Average body weight in gm. Initial	After 2 wks	After 4 wks	After 6 wks	Average gain	"t" test**	Total gain in weight over total feed intake (%)
15	None[1]		165.6±4.7*	235.4	465.4	620.4±23.8	454.8 ± 20.8		19.5
15	Ext. of viscera[2]	2.8	166.9±5.5	319.1	475.9	623.2±16.8	456.3 ± 14.4		20.2
14	Adsorbed fraction[3]	0.27	166.5±5.2	304.1	510.3	661.3±12.1	494.8 ± 13.9	1.6	18.6
15	Non-adsorbed fraction[4]	1.35	166.3±5.5	291.6	441.9	601.9±20.0	435.6 ± 15.0		17.2

1) To equalize the protein-content, soybean at a 3.5 per cent level was added. 2) Moisture, 44.8%; on dry basis, crude protein, 47.0%; NaCl, 24.8%. 3) Moisture, 45.3%; on dry basis, crude protein, 39%; NaCl, 19.7%. 4) Moisture, 45.9%; on dry basis, crude protein, 48.1%; NaCl, 46.2%. * Standard error of the mean. ** $n=28$, $c=0.05$, $t_0=2.048$.

Birds were fed basal diet A. The basal diet was supplemented with radish leaves at a 25～30 per cent level.

り，増加体重比率は基本区の 100 に対し 103，非吸着物区は最も劣り 96 であつた。内臓液化物区は第 4 週迄は最も優り，増加体重比率は基本区の 100 に対し 114 であつたが，以後は基本と殆んど差が認められなかつた。次に試験開始後 6 週目に解剖した結果は Table 3 に示した通りで，吸着物区が総ての点に於て発育良好で，他区よりも優れている。注目すべき点は非吸着物区の成績であつて，生長度（生体重，骨長）の

Table 3. Response in several organs of chicks to the extract of viscera adsorbed fraction of non-adsorbed fraction.

	None	Extract of viscera	Adsorbed fraction	Non-adsorbed fraction
Anterior hypophysis (mg)	5.7± 0.243*	5.5± 0.295	6.07± 0.230	6.03± 0.256
Testis (mg)	192.0±13.00	174.6±11.40	210.00±10.300	226.6 ±17.3
Humerus (cm)	6.2± 0.100	6.5± 0.067	6.50± 0.056	6.30± 0.077
Femur (cm)	6.5± 0.083	6.7± 0.059	6.80± 0.039	6.60± 0.086

* Standard error of the mean

点に於て他区より劣るにもかゝわらず，生殖腺発達度（脳下垂体前葉重量，睾丸重量）に於ては極めて優秀であつた。液化物区は基本区と大差ない成績であつた。以上の結果から吸着物は生長と生殖腺の発達を併行的に促進させる効果があるが，これに対し非吸着物は特に生殖腺の発達に対して選択的に作用するようにみえる。この点については今後なお追試し，脳下垂体前葉の組織学的精査と相俟つて検討したい。

Ⅳ. 総　　括

1. 乾物として 1.7 % のイタヤ貝内臓液化物を植物性蛋白飼料に添加したものを初生雛に 3 週間与えたところ，顕著な生長促進効果を示した。
2. 植物性飼料 100 g に内臓液化物 2.8 g より得た活性炭吸着物 0.27 g 或いは非吸着物 1.35 g を添加したものを，それぞれ孵化後 1 ケ月より 6 週間与えたところ，吸着物は生長促進効果を示したが，非吸着物は全く効果が認められなかつた。
3. 試験雛を解剖した結果，吸着物は生長と生殖腺の発達を併行的に促進するが，非吸着物は生殖腺の発達に選択的に作用するようにみえる。

本研究は昭和 26 年度の文部省科学試験研究費で支弁した。なお材料の調整並びに飼育に協力された菅原憲典，土井良平次郎，原田悠三，広渡一之，合屋正，行徳志信，松尾信一の諸氏に深謝の意を表す。

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貝類廃棄部の利用に関する研究 V.
鶏の生長及び産卵に及ぼすタイラギ内臓液化物の効果*

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貝類廃棄部の利用に関する研究—V.
鶏の生長及び産卵に及ぼすタイラギ内臓液化物の効果*

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Studies on Utilization of Wastes in Processing Shell-fish—V.
The Effect of Viscera-Extract of *Atrina pectinata japonica* on Chick Growth and Egg Production

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In a preceding report[2], it was shown that the addition of the viscera-extract of *Pecten laqueatus* at a 1.7 per cent dry weight basis gave a remarkable growth response of chicks and that APF in the viscera-extract was adsorbed on the activated charcoal from a slightly acidic solution.

The present paper deals with a study on the effectiveness of an autolyzed viscera-extract of *Atrina pectinata japonica* to the chick growth and the egg production, and further on the fractionation of the growth promoting principle. The results can be summarized as follows.

1. The addition to 100 g. of all-vegetable protein ration of a 2.8 g. portion of the dried autolyzed viscera and a 0.3 g. portion of an activated charcoal adsorbate which is equivalent to 2.8 g. of the dried autolyzate, resulted in a slight growth promotion on 35-day old chicks during six-week feeding as contrasted to a negative result obtained on addition of 1.35 g. of a non-adsorbed fraction (Table 1).

2. The we ght of anterior hypophysis and testis, and the length of humerus and femur increased in a linear proportion to the growth while the weight of thyroid gland inversely proportional (Table 2).

3. One-year old hens fed autolyzed viscera at a 2% dry weight basis for 100 days showed an egg production inferior to the control group. This is apparently due to a delay of starting egg production in the experimental group, because when compared as to the egg production of its highest production period (Feb. 7–March 30), a superior egg production in number as well as weight was observed in the experimental group (Table 3).

4. In the case of 5-month old hens fed an all-vegetable protein ration for 3 months prior to the experiment, the supplementation of the autolyzate at a 2% dry weight basis gave a remarkable response in egg production (Table 4).

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* 本研究の大要は昭和 27 年 4 月及び 28 年 4 月東京に於ける日本水産学会年会に発表した。

I 緒　　言

富山・杉原の方法[1]で製造したイタヤ貝内臓液化物は顕著な鶏雛の生長促進効果を示すこと，及び貝内臓液化物中の APF は微酸性で活性炭に吸着されることにつき既に報告した[2]。本研究では有明海に饒産するタイラギ内臓が鶏の生長及び産卵をどの程度促進するかについて検討し，更に有効成分の分別を試みた。

II 実 験 方 法

1. 試料の調製：1951年 11 月及び 1952年 2 月に有明海で採取したタイラギの貝柱のみを除いた内臓部に 50°C の湯を加え，塩酸で PH 4.5 とし 53～55°C で5時間液化し，一夜放置した後，濾過し，濾液を中和後 70°C 以下で減圧濃縮した。この内臓液化物は細目の笊で濾した程度であつたから，内臓液化物中の有効成分を分別するためには，液化物を水に溶かした後，先ず遠心して上澄液を分ち，他方不溶部を多量の水で数回洗つたものを遠心上澄液に合せて水溶部を調製した。有効成分の分別には水溶部に活性炭を加え PH 4.5 で吸着し，活性炭吸着部は稀苛性ソーダ水溶液（PH 9）で溶出した。溶出液（吸着物と略称する）及び活性炭に吸着されない濾液（非吸着物と略称する）はそれぞれ中和後 70°C 以下で減圧濃縮した。

2. 育雛試験：供試雛は 1952年 10 月 10 日に孵化した白色レグホン種の ♂ である。1 区 15 羽ずつとし屋外の竹格子底をもつバッタリで飼育した。11 月 14 日（孵化後 35 日）より 12 月 25 日まで飼養管理し毎週給餌前に体重を測定した。なお試験終了後試験雛を解体し，脳下垂体前葉，睾丸，甲状腺の重量及び上腕骨，大腿骨の長さを測定した。タイラギ内臓液化物区には Table 1 の脚註に示した基本飼料 100 g に対し乾物として 2.8 g の内臓液化物を，吸着物区及び非吸着物区には内臓液化物 2.8 g より得た吸着物 0.3 g 及び非吸着物 1.3 g をそれぞれ飼料 100 g に添加して与えた。なお緑餌として基本飼料の 50 % 相当量の大根葉を与えた。給餌には一種の paired feeding の方式をとり，給水は自由攝取とした。

3. 産卵試験：この試験は次に示すように 2 年鶏及び 1 年鶏について行つた。Table 3 に示した試験Aでは1950 年春孵化した白色レグホン種の内で 1 年間の産卵検査の結果，産卵数 200～220 個を示したもののみを選び，5 羽を 1 区として，1951 年 12 月 4 日より 1952 年 3 月 20 日まで飼養管理した。なお供試鶏は実験開始迄は Table 3 の脚註に示した魚粉を含む飼料で飼育した。Table 4 の試験Bでは 1951 年 8 月 23 日に孵化した白色レグホン種の ♀ 雛を脚註に示した魚粉を含まない基本飼料及び緑餌で 3 ケ月間飼育した後，発育度の平均した健康雛を選び，8 羽を 1 区として，1952 年 1 月 22 日より 3 月 20 日まで飼養管理した。いずれの試験に於いても試験鶏は戸外の三階作りの竹格子底をもつバッタリに収容し，試験区には基本飼料に乾物として 2 % のタイラギ内臓液化物を添加したものを与えた。他方，基本区には試験区の蛋白量に近似させるため大豆粕を 3.5 % 増補した。なお給餌量は 1 日 1 羽当り大約 120 g とした。trap nest の準備がなかつたので，各個体の産卵数は測定できなかつたが，1 区の総産卵数と 2 月 7 日より 3 月 20 日までの卵重量を測定した。

III 結 果 及 び 考 察

1. 鶏雛の生長に及ぼす効果

育雛試験の結果は Table 1 に示す通りで，タイラギ内臓液化物区は毎週最大の体重を示し，第 6 週に於ける増加体重比率は基本区の 100 に対し 110 であつた。吸着物区も僅かに優れた生長を示し，その増加体重比率は 104 であつたが，これに反し非吸着物は全く効果が認められなかつた。内臓液化物及び吸着物が顕著な効果を示さなかつた原因は，恐らく一種の paired feeding の型式をとつたためであろう。次に試験終了時の脳下垂体前葉，睾丸，甲状腺の重量及び上腕骨，大腿骨の長さを計測した結果は Table 2 に示す通りである。脳下垂体前葉は体重に正比例して増減している。しかしこの程度の差から機能の相違を推察することは危険である。睾丸は液化物区が最大，基本区がこれに次ぎ，吸着物区及び非吸着物区は略同じく最低である。第 4 報[2]に述べたように，イタヤ貝内臓の非吸着物は生殖腺の発達に対し撰択的に働くことが認められたが，タイラギ内臓の非吸着物は同様な効果を示さなかつた。この原因については次のように考えられる。生殖腺はその発達の或時期以後に初めて貝内臓非吸着物中の物質に鋭敏に反応し，それ以前は感受性を殆んど持たないものとも考えられるし，また加藤等[3]の研究によれば雛の睾丸は孵化後 3～4 ケ月の頃急速

Table 1. The growth response of chicks to viscera-extract of *Atrina pectinata japonica* and to fractions adsorbed and non-adsorbed on activated charcoal (Nov. 14 '52–Dec. 25 '52)

No. of chicks	Supplements	g. of supplements per 100 g. diet (Dry basis)	Average biweekly body weight in g.				
			Initial	After 2 wks	After 4 wks	After 6 wks	Average gain
13	Viscera-extract **)	2.8	174.5±7.7*	269.0	395.8	541.9±22.4	367.4±15.1
13	Adsorbed fraction †)	0.3	169.8±3.5	277.3	387.0	517.5±20.4	347.7±17.6
15	Non adsorbed fraction §)	1.3	172.4±7.2	276.1	369.7	500.0±29.2	327.6±24.1
14	None △)		171.1±4.1	268.0	374.3	503.7±16.6	332.6±14.9

* Standard error of the mean **) Moisture, 61.6 %; crude protein (on dry basis) 72.1 %. †) Moisture, 92.0 %; crude protein (on dry basis) 17.5 %. §) Moisture, 83.6 %; crude protein (on dry basis) 53.0 %. △) Basal diet used was composed of corn 30, soybean meal 25, rice bran 20, wheat bran 20, wheat 5, $CaCO_3$ 2, manganized salt 0.5. Basal diet was supplemented with the radish-leaves at a 50 per cent level.

Table 2. Weight of several organs of cockerels fed the extract of viscera, adsorbed fraction or non-adsorbed fraction

		Extract of viscera	Adsorbed fraction	Nonadsorbed fraction	None
Anterior hypophysis	(mg.)	5.3±0.5*	5.0±0.4	4.1±0.8	4.3±0.6
Testis	(mg.)	151.8±33.2	129.8±25.9	128.9±32.1	132.6±33.4
Thyroid gland	(mg.)	33.5±6.3	34.5±5.6	40.4±6.2	39.6±5.5
Humerus	(cm.)	5.96±0.22	5.96±0.20	5.74±0.22	5.86±0.18
Femur	(cm.)	6.37±0.25	6.38±0.22	6.08±0.23	6.24±0.19

* Confidence limit of the population mean.

に発達し，従つてこの頃は睾丸発達機転の一つのピークを示す時期であると断定出来る。そこで液化物区はたまたまそのピークに最も近い時期に迄発育し，他の三区は未だこれに遠いと考えれば液化物区以外の3区が大体同じ程度の重量を示すこともあり得べきことである。従つてもう少し試験期間を延ばせば，或は予期の結果が得られたかも知れない。甲状腺重量は体重と逆比例し体重の最も軽い非吸着物区が 40.4 mg で最大の重量を示した。しかし甲状腺重量の変化は腺自体の性質からみて，この程度の数値の差を以て或る意義

Table 3. Response in weight and production of eggs from one-year old hen fed the extract of viscera

No. of hen	Supplements	g. of supplements per 100 g. diet (Dry basis)	Dec. 4 '51 Mar. 20 '52	Feb. 7 '52 ~ Mar. 20 '52		
			Total number of egg produced	Total number of egg produced	Total egg weight g.	%
5	Viscera-extract *)	2	156	117	5915	120
5	None †)		200	100	4909	100

*) Moisture, 61.1 %; on dry basis, crude protein, 56.7 %; NaCl 18.0 %. †) To equalize the protein-content, soybean was added at a 3.5 per cent level.

Basal diet used was composed of corn 40, wheat bran 20, rice bran 20, soybean meal 20, NaCl 0.5, $CaCO_3$ 3. Basal diet was supplemented with the radish-leaves at a 30–60 per cent level. All the birds were fed animal-protein ration before the experiment. Animal-protein ration was composed of corn 50, wheat bran 15, rice bran 15, fish meal 20, NaCl 0.5, $CaCO_3$ 3.

を持たせる危険はさくべきである。上腕骨及び大腿骨長については数字的にも体重に正比例した変化がみられた。

2. 産卵率に及ぼす効果

（1） 二年鶏の産卵率向上効果：産卵数及び卵重量を計量した結果は Table 3 に示す通りで，全試験期間中の総産卵数をみると，タイラギ内臓液化物区は基本区より劣っているが卵重量を計量した2月7日より3月20日迄の産卵数及び卵重量では基本区のそれより著しく大であった。試験区の産卵数が対照に比し劣った原因は基本区では試験開始当日の12月4日には既に産卵していたのに対し，タイラギ内臓液化物区では1羽が1952年1月6日にようやく産卵し始めたような状態で，明らかに試験鶏の能力が不揃いであったためかと思われる。また供試鶏が試験開始迄に魚粉を含む飼料で飼育されていたことも，液化物の APF の効果が著明でなかつた原因であろう。

（2） 一年鶏の産卵率に及ぼす効果：産卵数及び卵重量を計量した結果は Table 4 に示す通りで，タイラギ内臓液化物区の産卵数及び卵重量は基本区を凌駕し，明らかに産卵率向上の効果を示した。

Table 4. The effect of supplementation of viscera-extract on weight and number of eggs of 5-month old hens

No. of hen	Supplements	Amt. of supplements per 100 g. diet Dry basis	Egg production†		Egg weight§	
			Total number	%	Total (g.)	%
8	Extract of viscera	2	151	125	5827	120
8	None*)		121	100	4843	100

*) To equalize the protein-content of the control feed to that of the experimental feed, soybean was added at a 3.5 per cent level to the basal diet given in foot-note ^) of Table 1. †) Jan. 22 '52-Mar. 20 §) Feb. 7 '52-Mar. 20.
All the birds were fed the basal diet shown in Table 3 for 3 months before the experiment and during the experiment.

Ⅳ 総　　括

1. 植物性飼料 100 g にタイラギ内臓液化物 2.8 g より得た活性炭吸着物 0.3 g 或は非吸着物 1.3 g を添加したものを，それぞれ孵化後 35 日より6週間与えたところ，内臓液化物及び吸着物は僅かに生長促進効果を示したが，非吸着物は全く効果が認められなかつた。

2. 試験雛を解剖した結果，脳下垂体前葉及び睾丸の重量並に上腕骨及び大腿骨の長さは生長と正比例したが，甲状腺重量は逆比例した。

3. タイラギ内臓液化物を基本飼料に乾物として 2% 添加したものを，白色レグホン種の一年鶏に 100 日間与えると，試験鶏の産卵開始の不揃いのためか総産卵数は基本区より劣った。しかし一般に産卵率が高くなる2月7日より3月30日迄の産卵数及び卵重量では基本区より著しく大であつた。

4. タイラギ内臓液化物を基本飼料に乾物として 2% 添加したものを試験開始前3ケ月間植物性蛋白飼料で飼育した白色レグホン種の一年鶏に与えると，顕著に産卵率を向上した。

終りに本研究は昭和26年度及び27年度の文部省科学試験研究費により実施したものである。試料の調製及び飼養管理に尽力された菅原憲典，奥山俊司，土井良平次郎，行徳忠信，佐伯種敏の諸氏に深謝の意を表する。

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煉製品製造殘滓の利用に関する研究—I. 鶏の生長及び産卵に及ぼすアラ液化物の効果*

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Studies on Utilization of Trimmings Wasted in Manufacturing Cooked Fish Paste — I.

Effect of solubilized trimmings on chick-growth and egg production.

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Only about 30 per-cent of fish body are ordinarily utilized for the preparation of the cooked fish paste, "Kamaboko". It can be estimated from a recent statistics that the trimmings annually ammounted to approximately 280,000 tons.

The present study has indicated that the trimmings can be stabilized by the liquefaction procedure proposed by T. TOMIYAMA and T. SUGIHARA[1]. Data have been presented supplementation of a solubilized trimmings at a level of 4.5 per-cent on dry basis to an all-vegetable protein diet resulted in a marked significant chickgrowth response. From its addition at a level of 2.9 per-cent dry basis to an all-vegetable protein diet resulted in a marked response in egg production of one-year old as well as two-years old hens and also an earlier start of egg production.

I. 緒 言

わが国では 1953 年の統計によれば, 年間約 4,500 万貫の煉製品が製造されている。これに用いている原料魚は約 11,600 万貫である。煉製品製造の残滓 (以下アラと略称する) は原料魚の約 60～70 %に達するから, 年間約 7,500 万貫のアラが得られている筈である。現在この多量のアラの一部を, 飼料及び肥料に用いてはいるが, 非常に腐敗し易いので特に夏季は完全に利用されていない。それ故にこれを安定化して蛋白資源の確保に資する目的をもつて本研究に着手した。

従来アラが養鶏飼料として適していることは経験的に認められているが, 鶏の生長及び産卵に及ぼすアラの効果についての研究報告はない。依つて著者等は先づ竹輪製造の際に得られるアラの安定化を試み, 更に安定化製品が鶏の生長及び産卵率にどの程度有効であるかについて検討した。その結果アラは富山の液化法[1]で安定化することが出来, 且つアラ液化物は鶏の生長促進, 産卵率向上及び産卵開始期の短縮等に著るしい効果を示すことを明かにした。

II. 実 験 方 法

A. アラの安定化: 日本水産K・K戸畑工場で, 竹輪製造の際に得た残滓 150 kg に 50 L の水と適量の

1955 年 5 月 30 日受理

* 本研究の大要は昭和 28 年 4 月 東京に於ける日本水産学会年会に発表した。

塩酸を加えてpH 4.5 とし，50～55°C で液化して濾過し，濾液を中和後減圧濃縮するとシラップとして 43.5 kg，乾物として 23 kg を得た。そのシラップの化学的組成は Table 2 に示す通りであつた。

Table 1. Composition of basal diet.

Kinds of basal diet	Corn	Soybean meal	Rice bran	Wheat bran	Wheat embryo	Fish meal	NaCl	$CaCO_3$	Manganzed salt †
A*	51.5	25	10	—	10	—	—	3	0.5
B*	46.5	30	10	—	10	—	—	3	0.5
C**	40.0	20	20	20	—	—	0.5	3	—
D**	50.0	—	15	15	—	20	0.5	3	—

* Folic acid 0.4 mg, 2-methyl-naphthoquinone 0.1 mg, lecithine lg, V.A. 2000 I.U., per 100 g diet, were added.

** Both diets were supplemented with fresh radish- and *Lactia indica*-leaves at 30–60 per-cent level.

† 96 g NaCl+6 g $MnSO_4$.

B．**育雛試験**： 試験区には Table 1 に示した基本飼料Aの 100 g に対し，乾物として 1.5 g，3 g，4.5 g のアラ液化物を小量の水にとかしてよく飼料に混じ，対照には基本飼料Aのみを与えた区の外に，アラ液化物 4.5 %区と粗蛋白量を同量とするため，大豆粕を 5 %増補した区を設けた。給餌及び給水は自由摂取とした。供試雛は 1952 年 7 月 25 日に孵化した白色レグホン種の♂である。100 羽から体重のそろつた強健な雛 50 羽を選択して 10 羽を 1 区とし，室内においた金網底をもつバッタリーに収容した。8 月 2 日より 8 月 23 日まで飼養管理し，毎週給餌前に体重を測定した。

C．**産卵試験**： この試験は次に示すように，2 年鶏及び 1 年鶏について行つた。試験（1）では，1950 年春孵化した白色レグホン種の内で 1 年間の産卵数 200～220 個を示したもののみを選び，5 羽を 1 区として 1951 年 12 月 4 日より 1952 年 3 月 20 日まで飼養管理した。なお供試鶏は実験開始迄は Table 1 に示した基本飼料Dで飼育した。試験（2）では 1951 年 8 月 23 日に孵化した白色レグホン種の♀雛を魚粉を含まない基本飼料C及び緑餌で 3 ケ月間飼育した後，発育度の平均した健康雛を選び，8 羽を 1 区として 1952 年 1 月 22 日より 3 月 20 日まで飼養管理した。いづれの試験に於ても，鶏を戸外の三階造りのバッタリーに収容し，試験区には基本飼料Cに乾物として 2.9 %のアラ液化物を添加し，試験区の蛋白量に近似させるため大豆粕を 3.5 %増補した基本区と対比した。なお風乾飼料の 30～60 %の大根葉或いは龍舌菜を混和して給与した。給餌量は 1 日 1 羽当り風乾飼料大約 120 g とした。Trap nest の準備がなかつたので，各個体の産卵数は測定できなかつたが，1 区の総産卵数と 2 月 7 日より 3 月 20 日までの卵重量を測定した。また試験（2）では産卵開始期をも記録した。

Ⅲ．結果及び考察

A．鶏雛の生長に及ぼす効果

各区の平均体重の増加は Table 2 に示す通りで，第 3 週目に於ける結果では体重増加比率は基本A 100 に対しアラ液化物 1.5 %添加区 96，3 %添加区 115，4.5 %区 126 であつたが，大豆粕を増補した基本 B100 に対しては 1.5 %区 89.5，3 %区 106.7，4.5 %区 117 であつた。即ちその投与量が多くなるにしたがつて生長促進効果が大となる。蛋白量の近似した対照と比較すると，4.5 %の投与量のものの効果が最も顕著で，基本Bの増加体重と 4.5 %区のそれとの差を検定した結果は 5 %の危険率で有意の差が認められた。

摂取飼料 100 g に対する増加体重即ち飼料効率は Table 2 に示す通りで，アラ液化物 1.5 %添加区では対照との差が認められなかつたが，3 %区及び 4.5 %区の飼料効率は対照区のそれより著るしく大きかつた。

Stokstad 氏等[1]の研究によれば，鶏の生長に必要な B_{12} 量は 100 g の飼料に 1.5～3.0 g である。依つて著者等はアラ液化物中の B_{12} 量を Hutner の Euglena 法で測定した結果，Table 2 に示すようにアラ液化物の乾物 100 g 中には僅かに 0.665 μg の B_{12} を含むにすぎなかつた。従つて飼料 100 g 中に添加した乾物 4.5 g 中の B_{12} は 0.03 μg で，鶏の生長には全く無効な量である。依つてアラ液化物のこのような著るしい効果は，アラ液化物中の B_{12} 以外の未知 AFP の存在によるものと考えて差支えない。

B．産卵率に及ぼす効果

Table 2. Growth response of chicks to solubilized trimmings.
(Aug. 2 '52～Aug. 23)

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Basal diet Kinds	Basal diet B_{12} mμg%	Basal diet Crude protein %	Average weekly body wt. in gm Initial	After 1	After 2	After 3	Average gain	"t" test[2]	Total gain in weight over total feed intake (%)
10	Basal diet A		A		18.0	59.5 ±1.8*	92.3	122.7	148.5 ±8.4	89.0 ±7.4		29.2
9	Basal diet B		B		19.7	58.3 ±1.7	91.0	120.8	154.1 ±4.5	95.8 ±4.9		29.1
9	Solubilized trimmings[1]	1.5	A	7	18.6	60.0 ±1.5	96.2	121.5	145.6 ±7.2	85.6 ±6.7		28.2
10		3.0	A	15	19.2	60.4 ±1.3	95.2	129.1	162.4 ±7.3	102.0 ±6.6		35.6
10		4.5	A	30	19.8	60.4 ±1.5	97.3	130.9	172.5 ±4.9	112.1 ±5.4	2.23	36.0

1) Moisture 47.1 %, crude proiein (dry basis) 75.4 %, NaCl (dry basis) 8.1 %.
2) Test for the difference between 4.5 % group and basal diet B group. $n=17$ $0.05\cdots\cdots t_0=2.11$.
* Standard error of the mean.

1) **二年鶏の産卵率向上効果**： 産卵数及び卵重量を計量した結果は Table 3 に示す通りで，アラ液化物区の産卵率は著しく高く，産卵比率は基本の 100 に対し 131 を示し，卵重量比率は基本の 100 に対し 116 を示した。

Table 3. Response in weight and production of eggs from two-years old hen fed the solubilized trimmings.

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Crude protein per 100 g diet.	Egg production § Total number	Egg production § %	Egg weight (g) † Total	Egg weight (g) † %
5	Solubilized trimmings	2.9	21.2	261	131	5710	116
5	None *	—	20.9	200	100	4909	100

* To equalize the protein content, 3.5 per cent level of soybean were added.
§ Dec. 4 '51～Mar. 20 '52. † Feb. 7 '52～Mar. 20.
All the birds had been fed the basal diet D before the experiment and were fed the basal diet C during the experiment.

2) **一年鶏の産卵開始期並に産卵数に及ぼす効果**： 産卵数及び卵重量を計量した結果及び産卵開始期は Table 4 に示す通りで，アラ液化物区は基本区より 1 週間前から産卵し始め，産卵数及び卵重量でも基本区に比し優る結果を示した。

Table 4. Response in weight and number of egg produced and date of start of egg production from one-year old hens fed the solubilized trimmings.

No. of chicks	Supplements	Amt. of supplements per 100 g diet. Dry basis	Crude protein per 100 g diet.	Egg production § Total number	Egg production § %	Egg weight (g) † Total	Egg weight (g) † %	Date of start of egg production
5	Solubilized trimmings	2.9	21.2	144	119	5331	110	Jan. 30
5	None *	—	20.9	121	100	4843	100	Feb. 6

* To equalize the protein-content, 3.5 per cent level of soybean were added.
§ Jan. 22 '52～Mar. 20. † Feb. 7 '52～Mar. 20.
All the birds were fed the basal diet C during the experiment and for 3 months before the experiment.

以上二つの試験結果より，アラ液化物は明らかに産卵率向上効果を示すことが実証しえられた。

Ⅳ. 総　　括

1. アラ液化物は投与量 1.5～4.5 %の範囲では増量する程，雛の生長促進効果が大であつた。特に投与量4.5%では飼料の蛋白量を近似させた基本区と5%の危険率で有意の差が認められた。

2. アラ液化物中の B_{12} 含量を Hutner の Euglena 法で測定した結果，乾物 100 g 中に 0.665 γ g 存在し，投与量 4.5 g のアラ液化物中の B_{12} は僅かに 0.03 μg となる。依つてアラ液化物の効果は B_{12} 以外の未知 APF の効果と考えられる。

3. アラ液化物を基本飼料に乾物として2.9%添加したものは，白色レグホン種の二年鶏の産卵率を著るしく向上した。

4. アラ液化物を基本飼料に乾物として2.9%添加したものを一年鶏に5ケ月間与えると，産卵開始が無添加区に比し約1週間早くなり，また産卵数は約 19 %多かつた。

終りに，本研究は昭和 26 年及び 27 年度の科学試験究究費により実施したものである。試料の調製及び飼養管理に尽力された管原憲典，奥山俊司，土井良平次郎，佐伯種敏の諸氏並びに材料を提供された日本水産株式会社に深謝の意を表する。

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煉製品製造殘滓の利用に関する研究…Ⅱ.
鶏の生長及び産卵に及ぼすアラ液化物の長期投與の効果*

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煉製品製造殘滓の利用に関する研究—Ⅱ. 鷄の生長及び産卵に及ぼすアラ液化物の長期投與の効果*

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Studies on Utilization of Trimmings Wasted in Manufacturing Cooked Fish Paste——Ⅱ. A long-term experiment on effect of solubilized trimmings on growth and egg production.

Tetuo Tomiyama, Yasuo Yone, Kazuyuki Hirowatari, Takeo Koyanagi, Shogoro Uchida and Hisao Tokuda

Chicks fed a diet supplemented with the solubilized trimmings at a 2 or 3 per cent dry basis level for 8 to 32 weeks showed a significant growth response, (Table 1 and 2). The difference in body weight between the chicks fed the solubilized trimmings and the control chicks fed only all-vegetable protein ration was significant at a 5% level (Table 1). It is to be noted that earlier start of egg production has been found in the experimental group (Table 2). It has been shown that chicks which were raised on the ground without using battery-house gave an increase of 14 per cent in the growth rate after 9 weeks by feeding an all-vegetable protein ration with the solubilized trimmings at a 3 per cent level (Table 3).

Ⅰ 緒　　言

著者等[1]はアラ液化物が鷄の生長促進，産卵率向上，産卵開始期の短縮等に効果を有することにつき既に報告した。しかしこれらの実験は断片的に比較的短期間観察したものである。従つて本研究では孵化後数日より産卵開始期に至るまでの長期に亘つて育雛産卵試験を行つた。この研究結果によりこれ迄の著者等の結論の妥当性を確証しえたので，こゝに報告する。

Ⅱ 実 験 方 法

1. アラ液化物

供試したアラ液化物は日本水産戸畑工場で 1952 年 6 月及び 1953 年 3 月に竹輪製造の際にえられたアラを富山の液化法[1]で製造したものであつて，その化学的組成は Table 1 の脚註に示した。

2. 初生雛より産卵までの試験

供試雛は 1952 年 6 月 17 日に孵化した白色レグホン種の♀である。60羽から体重の揃つた強健な雛 20 羽を選び，10 羽を 1 区とし，6 月 23 日より 9 月 30 日迄飼育した。10 月 1 日に各区の平均体重に近い雛を

1955 年 7 月 18 日受理

* 本研究の大要は昭和 28 年 4 月東京に於ける日本水産学会年会に発表した。

5羽づゝ選び飼育した。なおC月23日より9月3日までは室内においた金網底をもつバッタリーに収容し，9月4日以降は戸外の金網底或は竹格子底をもつバッタリーで飼育した。なお体重は毎週給餌前に測定した。また各個体の産卵能力は不明であったから，トラップネストを用いて各個体の産卵開始日及び産卵数を求めた。

試験区には6月23日より9月30日迄は基本飼料に乾物として3%のアラ液化物を添加したもの，10月1日以後は乾物として2%を添加したものを与えた。なお基本区には試験区と蛋白量を近似させるため大豆粕を3%添加した。基本飼料は6月23日より9月30日迄はTable 1の脚註に示した基本飼料Aを用いた。9月4日より9月30日までは葉酸，ビタミン K_3 及びレシチンの投与を中止し，基本飼料の50%に相当する大根葉を与えた。10月1日以降は基本飼料Bに大根葉，龍舌菜を50%混和したものを与えた。水及び飼料は充分量投与し，自由摂取とした。

3. 育雛試験

供試雛は1953年10月8日に孵化した白色レグホン種の♀雛で，1区50羽とした。孵化後3週間は箱型電熱育雛箱で飼育し，その後は実際的な飼育法，即ち"地飼い"で飼育した。毎週給餌前に体重を測定した。試験区にはTable 3の脚註に示した基本飼料に乾物として3%のアラ液化物を添加し，対照区には試験区の蛋白量と近似させるために大豆粕を6.5%増補した。またいづれの区にも緑餌として基本飼料の15%相当量の大根葉を与えた。なお給水及び給餌は自由摂取とした。

Ⅲ 結果及び考察

A 生長及び産卵率に及ぼす効果

供試鶏10羽の内，試験区の1羽及び対照区の1羽が試験開始後8週迄に斃死し，また試験区の1羽は8

Table 1. Response of the growth to the solubilized trimmings.

No. of Chicks	Supplements	Amt. of supplements per 100g diet. Dry basis	Average 4-weekly body weight in gm.				"t" test**	Total gain in weight over total feed intake (%)
			Initial*	After 4 wks	After 8 wks	Average gain		
8	Solubilized trimmings †	3	41.1±1.7§	211.0	403.0±14.6	361.9±14.1	2.19	19.7
9	None	—	40.2±1.4	184.6	358.8±14.1	318.6±13.9		21.9

* The experiment was started on June 23, 1952. ** n=15, ε=0.05, t_0=2.131.
† V. B_{12} content, 352 mμg%; Moisture, 47.1%; On dry basis, crude protein, 75.5%; salt, 8.1%. Birds were fed the basal diet A which was composed, in per cent, of corn 51, soybean 25, rice bran 10, wheat embryo 10, $CaCO_3$ 3, manganized salt 0.5, folic acid 0.4mg, 2-methyl-naphtoquinone 0.1mg, lecithine 1g. § standard error of the mean

であつた。試験開始後第8週迄の各区の平均体重はTable 1に示してある。それをみると飼育開始後第4週（7月21日）の増加体重比率は基本の100に対し，アラ液化物区は118であり，第8週は113であつた。またアラ液化物区の増加体重と基本区のそれとの差に対するt検定の結果では，5%の危険率で有意の差が認められる。しかし飼料効率では両区の間に著るしい差は認められなかつた。

次ぎに10月1日以降供試した5羽の内，各区ともそれぞれ1羽づゝ試験期間中に斃死したので，4羽の全試験期間中の平均体重曲線及び各個体の体重をFig. 1及びTable 2に示した。アラ液化物区の生長は3週より基本区の生長を凌駕し，飼育開始後35日後（7月28日）の増加体重比率は基本の100に対し116，114日後（10月15日）は118であつた。なお生長の極限に近づいた224日後（1953年2月1日）は109で，なお体重差が認められた。4羽の産卵成績及

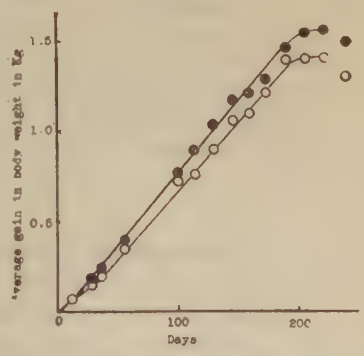

Fig. 1. Growth response to supplementation of solubilized trimmings.
● 2~3% solubilized trimmings
○ no supplement

Table 2. Response of the growth and egg production to the solubilized trimmings.

Supplements	Chicks No.	Body weight in gm. Initial	after 35 days	after 114 days	after 224 days	Jan. 6—July 31, '53 6-weekly production of egg 0-6	6-12	12-18	18-24	24-30	Total number	Date of start of egg production
Solubilized trimmings 2~3%	7058	36	256	995	1750	4	19	35	20	0	404	Feb. 7
	4719	42	320	840	1600	13	13	32	28	14		Jan. 21
	4740	43	244	840	1350	18	17	35	25	14		Jan. 12
	8653	49	240	1090	1650	18	19	30	29	21		Jan. 7
None*	4607	38	213	790	1500	18	23	34	33	13	394	Jan. 6
	4736	35	230	800	1600	16	20	32	35	7		Jan. 9
	4667	42	253	900	1300	0	14	32	30	18		Mar. 2
	8046	43	218	700	1400	0	12	29	21	7		Mar. 2

* To equalize the protein-content, 3 per cent of soybean were added.
Birds were fed the basal diet A from June 23 to Sep. 30 and the basal diet B after Nov. 1. Basal diet A was shown in Table 1. Basal diet B was composed, in per cent, of corn 47, rice bran 10, wheat bran 10, soybean 30, $CaCO_3$ 3. Basal diet B was supplemented with radish- and *Lactica indica*-leaves at a 50 per cent level.

び産卵開始日は Table 2 に示す通りで，アラ液化物区はいづれも2月初旬までに産卵を開始したのに対し，基本区は4羽の内，2羽が3月に産卵を開始した。従ってアラ液化物区の産卵開始後6週間の産卵数は基本区を凌駕していた。一般に産卵率が高くなる6週（2月16日）以後は差がなくなり，全試験期間の産卵率では顕著な差が認められなかつた。

B 生長に及ぼす効果

従来の生長試験は少数羽の雛について行つたが，本研究では1区に50羽の雛を用い，実際的な飼育法，即ち“地飼い”で飼育した。育雛試験の結果は Table 3 に示す通りで，第5週まではアラ液化物区の生長は基本区のそれより僅かに劣っていたが，第6週以後基本区の生長を凌駕し，第9週に於ける増加体重比は基本区の100に対し114を示した。

Table 3. Response of the growth to the solubilized trimmings.
(Oct. 8 —Dec. 10, '53)

No. of chicks	Supplements	Amt. of supplements per 100g diet Dry basis	Body weight[1] in gm Initial	After 3 wks	After 6 wks	After 9 wks	Average gain	Total gain in weight over total feed intake (%)
50	Solubilized trimmings	3.0	38	101.1	244.4	437	399	27.8
50	None[2]	—	38	105.0	241.6	387	349	24.4

1) The body weight was determined not indivdually but as a group. 2) To equalize the protein content, 6.5 per cent of soybean were added.
Basal diet used was composed, in per cent, of corn 52, soybean meal 30, rice bran 15, $CaCO_3$ 2.5, NaCl 0.5. Birds were raised on the ground without using battery house.

Ⅳ 総　　括

1. アラ液化物を乾物として3%添加した飼料を孵化後1週間目から8週間投与すると，著明な生長促進効果がみられ，対照区に比し5%の危険率で有意の差が認められた。

2. 乾物として2～3%のアラ液化物を添加した飼料を白色レグホン種の孵化後1週間目より産卵開始迄約32週間投与すると，著明な生長促進効果がみられた。また対照区より早く産卵を開始し，産卵率では産卵開始後6週間の産卵数に差がみられた。

3. 50羽の白色レグホン種の雛に乾物として3%のアラ液化物を添加した飼料を孵化直後より9週間投与

すると，顕著な生長促進効果がみられた。

本研究は昭和 27 年度及び 23 年度の文部省科学試験研究費を支弁した。なお試料の調製及び動物の飼育に協力された土井良平次郎、佐伯種政、久米利治の諸氏並びに材料を提供された日本水産株式会社に深謝の意を表する。

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煉製品製造殘滓の利用に関する研究 — Ⅲ.
換羽期の産卵率低下の防止に対するアラ液化物の効果*

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Studies on Utilization of Trimmings Wasted in Manufacturing Cooked Fish Paste——Ⅲ. Effectiveness of solubilized trimmings to prevention of lowering of the egg production in molting season.

Tetuo TOMIYAMA, Yasuo YONE, Kazuyuki HIROWATARI, Takeo KOYANAGI, Shogoro UCHIDA and Hisao TOKUDA

It has alraedy been reported that the solubilized trimmings at a 3 per cent level is effective to increase the egg production[1]. All the experiment, however, have been made in seasons favorable to the egg production. The present experiment has been undertaken to see whether or not the trimmings will show preventative effect to the reduction of egg production in autumn. It has clearly been shown that a decrease of egg production during the molting season could be prevented by the addition of the solubilized trimmngs at a 2.9 per cent level.

Ⅰ 緒 言

さきに著者等[1]はアラ液化物が産卵率の向上，産卵開始期の短縮等に効果を有することを確認した。しかし産卵試験は産卵率の最も高い期間に行つたものである。従つて産卵率の低下する換羽期に於ける産卵にどの程度の効果を示すかを知ることは産業的に重要である。更にまた養鶏用の所謂完全飼料には一般に15～20%の魚粉を必要とするが，現在の魚粉の価格ではかように多量を使用することは経営上困難である。従つて他の適当な APF の給源を用うることによつて魚粉の使用量を低減できれば，経営上極めて有利となるに違いない。依て著者等は上記のような見地から本研究を行つた。

Ⅱ 実験方法

1. アラ液化物

供試したアラ液化物は日本水産戸畑工場で 1952 年 6 月に竹輪製造の際にえられたアラを富山の液化法[1]で製造したものであつて，その化学的組成は Table 1 の脚註に示してある。

2. 産卵試験

試験鶏は 1952 年 2 月 11 日及び 23 日に孵化した白色レグホン種で，試験開始前 2 ケ月間は一般の成鶏用飼料で戸外に飼育したものの内，体重 1,300～2,000g の強健なもの 20 羽を選び，1 区に 5 羽を用いた。竹格子底にした戸外のバッタリーに収容し，7 月 8 日より試験飼料の投与を開始し，7 月 12 日には各区と

1955 年 7 月 18 日受理

* 本研究の大要は昭和 28 年 4 月東京に於ける日本水産学会年会に発表した。

も約半数が産卵を開始したので産卵数の測定を始め，10 月 31 日迄経続した。なお各個体の産卵能力は不明であったから，Trap nest を用いて各個体の産卵数を求め，駄鶏は産卵率の計算から除外した。

Table 1. Composition of diets used.

Groups of expt.	Fish meal	Corn	Wheat bran	Soybean meal	Salt	Solubilized trimmings	Crude protein %
Fish meal	20	40	40	—	0.5	—	19.1
Solubilized trimmings 2.9% +Fish meal 10%	10	45	40	—	—	5	16.6
Solubilized trimmings 2.9% +Fish meal 5%	5	45	40	5	—	5	16.7
Soybean meal	—	40	40	24	0.5	—	21.5

* $V.B_{12}$ content, 352m·g.%; Moisture, 47.1%. On dry basis, crude protein, 75.5%; salt, 8.1%. All the diets were supplemented with radish- and *Lactica indica*-leaves at a 17 per cent level.

試験区としては魚粉 20% 含有の標準区，魚粉を 10% としてアラ液化物 2.9% (乾物として)添加した区魚粉を 5 %としてアラ液化物 2.9%添加した区及び大豆粕 24% 含有の APF を含まない区を設けた。アラ液化物は適量の水にとかし試験配合飼料中に均一に混和して与え，投餌量は各区とも 1 日 1 羽当り大約120g とした。なお各試験区の蛋白含量を近似させるためにアラ液化物 2.9%—魚粉 5 %区のみに大豆粕を 5 %添加した。また各試験区とも大根葉及び龍舌菜を飼料の 17% 相当量与えた。各試験区の飼料配合率及び蛋白含量の詳細は Table 1 に示してある。

II 結果及び考察

産卵試験の結果は Table 2 及び Fig.1 に纒めた通りである。産卵効果を時期的に解折するために，10 日

Table 2. Biweekly change of egg production in each experimental group.
(July 12～Oct. 21,'52)

Supplements	Chicks No.	Biweekly production of egg 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	Total number (%)	8-14wks egg production number (%)	Date of start of egg production	Days of no egg production
Fish meal 20%	923	10	10	9	8	8	5	4	11	195 (43.5)	55 (32.7)	VII/ 9	0
	924	10	9	7	0	0	2	1	0			VII/ 8	73
	926	0	10	9	11	11	12	11	10			VII/26	0
	927	6	9	10	1	1	0	0	0			VII/14	68
Solubil. trimmings 2.9%+ Fish meal 10%	602	14	6	12	10	12	2	9	10	254 (56.6)	83 (49.4)	VII/ 8	12
	903	11	10	7	10	9	6	9	9			VII/11	0
	904	0	8	9	11	10	11	9	7			VII/23	0
	905	0	7	11	13	6	0	0	6			VII/30	39
Solubil. trimmings 2.9%+ Fish meal 5%	912	0	0	7	9	8	0	0	0	229 (51.1)	91 (54.1)	VIII/ 9	46
	915	6	7	6	7	8	0	4	11			VII /19	26
	916	13	10	9	9	13	12	12	11			VII / 8	0
	917	0	3	8	11	10	12	12	11			VIII/ 4	0
Soybean meal 24%	939	11	8	1	0	0	0	0	7	236 (42.1)	49 (23.3)	VII /11	75
	940	12	13	12	8	0	2	5	12			VII / 9	24
	941	11	12	11	10	6	0	1	0			VIII/14	46
	942	0	2	8	3	6	9	5	12			VIII/ 7	12
	943	0	2	11	11	8	1	6	10			VII / 6	27

The renewal of feather was observed to take place in each group from 8— to 14—weeks, and the degree of the renewal ran parallel with decrease in egg production.

間以上産卵を中止した日数（産卵中止日数と仮りに呼ぶ），全試験期間中の産卵率及び全般に換羽が観察された期間中（9月6日より 10 月 17 日迄）の産卵率を算出した。先づ産卵中止日数について比較すると，アラ 2.9%一魚粉 10% 区が 51 日で最も小さく，アラ 2.9%一魚粉 5 %区が 72 日，魚粉 20% 区が 141 日大豆粕 24% 区が 184 日であつて，アラ液化物の効果が顕著であつた。全試験期間中の産卵率はアラ2.9%一魚粉 10% 区が最高で，アラ 2.9%一魚粉 5 %区がこれに次ぎ，魚粉 20% 区及び大豆粕 24% 区は遙かに劣り，第1位のものの 74 % に相当した。換羽期間，即ち試験開始後 8 週より 14 週までの産卵率をみると，アラ 2.9%一魚粉 5% 区が最も優り，アラ 2.9%一魚粉 10% 区がこれに次ぎ，魚粉 20% 区は蛋白含有量が前2者に比し多少多いに拘らず産卵率は遙かに劣り，また蛋白含有量の最も多い大豆粕 24% 区は極めて低値で，第1位のものゝ 43% にしか達しなかつた。こゝに注目される点はアラ液化物を添加した区はいずれも換羽期間の産卵率は全期間の産卵率と大差がなかつたのに対し，魚粉区及び大豆粕区では換羽期間の産卵率は全期間の産卵率より著しく低下したことである。但し完全区であるべき魚粉区の成績が劣つたのは，良質の魚粉を選ぶように注意したので，恐らくは Stick water が充分に圧搾除去された魚粉を使用したことに原因したのであろう。

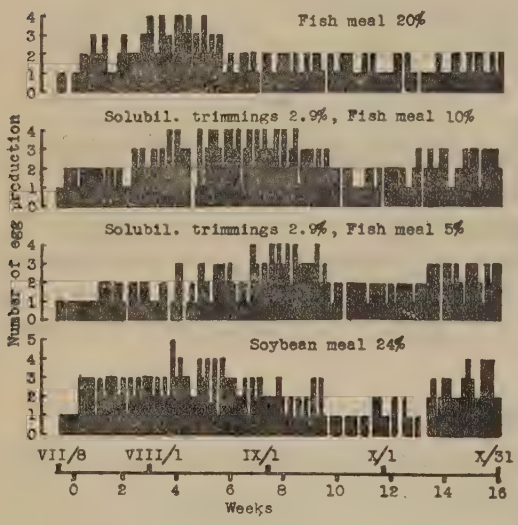

Fig. 1. Change in egg production during molting season.

Ⅳ 総　　括

標準の完全飼料の魚粉 20% を 10% 及び 5 %に減じたものにアラ液化物を乾物として 2.9% 添加した飼料で，1952 年 2 月孵化の白色レグホン種を産卵開始直前の 7 月初めより 10 月未迄 16 週間飼養して，産卵率の変動を比較検討した結果によると，換羽期の産卵率低下が著明に防止された。

本研究は昭和 27 年度の文部省科学試験研究費で支弁した。本研究の遂行に多大の補助を与えられた原田悠三，管原憲典，奥山俊司，土井良平次郎，久米利治の諸氏並びに材料を提供された日本水産株式会社に深謝の意を表する。

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煉製品製造殘滓の利用に関する研究—Ⅳ. 殘滓液化物による産卵上昇効果に関する実地試験

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Studies on Utilization of Trimmings Wasted in Manufacturing "Kamaboko", a Cooked Fish Cake——Ⅳ. A large-scale experiment on the effectiveness of solubilized trimmings on egg production.

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In all the experiments on egg production which have hitherto been carried out[1-3], use was made of rather small number of hens and of the battery house.

The present study has been carried out on one-year old hen without using the battery house and under conditions as practical as possible.

The results obtained can be summarized as follows :

1. The supplementation of the solubilized trimmings at a 5 % dry basis level gave more egg production as compared with that of fish meal at a 10 per cent level.
2. The partial replacement of the fresh trimmings with the solubilized trimmings yielded more or less a favorable effect on egg production.
3. The supplementation of the solubilized trimmings at a 2.5 per cent dry basis level gave egg production similar to the control group, while a little increase was observed from July to September.

Ⅰ 緒 言

著者等[1)2)3)]は 1951 年秋より 3 年間鶏の生長及び産卵率に及ぼすアラ液化物の効果について検討した結果、アラ液化物の顕著な APF 効果を認めた。これまでの産卵試験は全て少数羽の鶏を用い、バッテリーで飼育して行った。従ってバッテリーを用いず実際的な飼育法で産卵試験を行い、その効果を検討することは本研究の完成に当り意義あることと考え、福岡県内の養鶏業者と協同して産卵数に及ぼすアラ液化物の効果を魚粉、生魚屑(アラ)或いは大豆粕の効果と比較した。本試験は長期間に亘り実施する予定であったが、1953 年 6 月の大水害により養鶏業者の大半が多少の被害を受け、中止した業者があったことは残念であった。しかしその結果は本研究の成果の実用化に有益な指針を含んでいるので、以下にその概要を報告する。

Ⅱ 実 験 方 法

1. アラ液化物の製造

本試験に用いたアラ液化物は日本水産戸畑工場で竹輪製造の際に得られた生魚屑(採肉残渣、一般にアラ

1955 年 7 月 30 日受理

と称す）を富山の液化法[1]で安定化したもので，水分 50 %，粗蛋白 42.3 % のものである。

2. 魚粉との比較試験

本試験は次に示すように2回行った。試験（A）では Table 1 の脚註に示す基本飼料にアラ液化物を乾物として 5 %（粗蛋白 4.2 %）添加し，対照の魚粉区には基本飼料に 10 %（乾物として約 8.5 %，粗蛋白約 5 %）の魚粉を添加した。供試鶏は 1952 年 2 月 10 日に孵化した白色レグホン種の2年鶏である。アラ液化物区には 32 羽，魚粉区には 37 羽を用いた。飼育室としてはアラ液化物区のものは2坪の運動場を有する1坪の室4個に収容し，魚粉区は6坪の運動場を有する 4坪の室1個に収容し，1953 年2月より4月迄飼養管理した。

試験（B）の添加量は試験（A）と同じであるが，基本飼料は Table 1 の脚註に示したように試験（A）で用いたものとは多少異っていた。供試鶏は 1952 年 3 月 29 日に孵化した白色レグホン種の2年鶏である。いづれの区もそれぞれ1坪の運動場を有する1坪の室5個に飼育し，1953 年 2 月より 5 日まで飼養管理した。

3. 生魚屑との比較試験

本試験は次に示すように2回行った。試験（C）では試験区に Table 2 の脚註に示す基本飼料に乾物として 2.5 % のアラ液化物と 10 % の生魚屑を添加し，対照の生魚屑には 15 % の生魚屑を添加して比較した。供試鶏は 1952 年 2 月 23 日に孵化したロード，ロック及びニューハンプシヤ種の2年鶏で，飼育にはいづれの区も6坪の室を2個使用した。

試験（D）の試料添加量は試験（C）と同じである。供試鶏は 1951 年 3 月 20 日に孵化した白色レグホン種の3年鶏である。いづれの区もそれぞれ12坪の運動場をもつ10坪の室で飼育した。

4. 大豆粕との比較試験

本試験は福岡県種鶏場で行った。試験区には日本配合飼料 K.K. の配合飼料（組成不明）に乾物として，2.5 % のアラ液化物を添加し，対照区には試験区と蛋白量を近似させるため大豆粕を 4 % 添加した。供試鶏は1952年3月5日に孵化した白色レグホン種の2年鶏である。いづれの区もそれぞれ10坪の運動場をもつ6坪の室2個に収容し飼養管理した。

以上いづれの試験に於ても給餌量は1日1羽当り大約 120 g とした。

Ⅲ 結果及び考察

1. 魚粉との比較

産卵率は Talbe 1 に示す通りで，試験（A）の場合，試験開始時の産卵率は対照の魚粉区がアラ液化物区より優れていたが，以後はアラ液化物区が魚粉区を凌駕した。試験（B）の場合は試験（A）の場合と同じく

Table 1. The effect of supplementation of solubilized trimmings on egg production as compared with that of fish meal.

	Supplements	No. of hen[4]	Rate of egg production				Average rate of egg production
			Feb.' 53	March	April	May	
A[1]	Solubilized trimmings[3] 5 %	32	70.9	76.0	74.1	——	73.6
	Fish meal 10 %	37	74.7	74.5	70.8	——	73.3
B[2]	Solubilized trimmings 5 %	64	70.8	79.1	77.3	76.0	75.8
	Fish meal 10 %	55	68.6	80.3	72.4	74.0	73.8

1) Basal diet used was composed, in per cent, of a commercial ration A 40, barley 16, wheat bran 16, rice bran 8. The green leaves were supplemented at a 15 % level. 2) Basal diet used was composed, in per cent, of a commercial ration B 65, barley 19, wheat bran 10, rice bran 6. The green leaves were supplemented at a 15 % level. 3) Moisture 50 % ; Crude protein 42.3 % on dry basis. 4) Single comb white Leghorn.

試験開始後2ケ月間はアラ液化物区の産卵率は魚粉区のそれと殆んど差がなかったが，以後2ケ月間はアラ液化物区の産卵率が魚粉区より大であった。

これの試験では，アラ液化物に添加した蛋白量は魚粉区のそれより少かったに拘らず，産卵効果が優っているのは明かに APF の多量の供給によるものと考えられる。

2. 生魚屑との比較

アラ液化物の産卵効果とアラ液化物の原料である生魚屑の効果との比較試験の結果は Table 2 に示す通り

Table 2. The effect of supplementation of solubilized trimmings on egg production as compared with that of fresh trimmings.

	Supplements	No. of hen	Rate of egg production Feb. '53	March	April	May	June	July	Average rate of egg production
C[1]	Solubilized trimmings 2.5 % +Fresh trimmings 10 %	80	——	75.6	73.1	68.1	——	——	72.2
	Fresh trimmings 15 %	88	——	71.9	69.8	62.4	——	——	68.0
D[2]	Solubilized trimmings 2.5 % +Fresh trimmings 10 %	100	36.7	42.1	76.3	77.4	66.7	53.9	58.8
	Fresh trimmings 15 %	100	38.6	39.6	70.1	72.8	62.2	50.8	55.7

1) Basal diet used was composed, in per cent, of corn 20, wheat 20, barley 10, wheat bran 20, rice bran 20, rice hull 10. The group of hen was composed of Rhode 20, Prymouth Rock 45, and New Hamp- shire 15 or 23. 2) Basal diet used was composed, in per cent, of corn 25, wheat 10, barley 10, ground wheat 10, wheat bran 15, rice bran 20, barley bran 10. The green leaves were added at a 30 per cent level. Single comb white Leghorn was used.

で，試験 C の場合，全試験期間を通して常にアラ液化物区の産卵率は生魚屑区のそれを凌駕した。

試験 D の場合は，試験開始直後の産卵率では生魚屑 15 % 区がアラ液化物 2.5 %一生魚屑 10 % 区より優れていたが，以後はアラ液化物 2.5 %一生魚屑区 10 % 区の産卵率が生魚屑のみの対照区のそれを凌駕した。

3. 大豆粕との比較

産卵成績は Table 3 に示す通りで，全試験期間の産卵率はアラ液化物 2.5 % 区と基本区との間には差が認められなかった。月別の産卵率を詳細にみると，試験を開始してから5ケ月間は基本区が僅かに優れていたが，所謂生み疲れの時期（7月～9月）はアラ液化物区の産卵率が基本区のそれより高いことが注意される。

Table 3. The effect of supplemenntation of solubilized trimmings on monthly egg production.

Supplements	No. of hen[2]	Monthly rate of egg production Feb. '53	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Average rate of egg production
Solubilized trimmings 2.5 %	100	77.5	83.5	78.9	75.2	70.3	77.5	67.0	63.9	41.9	70.6
None[1]	100	76.0	80.1	81.5	78.8	71.7	70.8	65.5	59.5	42.4	69.5

1) To equalize the protein-content, soybean at a level of 4 per cent of the ration was added.
2) Single comb white Leghorn.
The basal diet used was a commercial ration A. The green leaves were added at a 40 per cent level.

Ⅳ 総　　括

一般の飼養管理法で飼育した2～3年鶏の産卵に及ぼすアラ液化物の効果について検討し，次の結果を得た。

1. 乾物として 5% のアラ液化物を添加した飼料で白レグの2年鶏を 1953年2月から 3～4 ケ月間飼養管理したところ，10% の魚粉を添加した飼料で飼育した場合に比し，時日の経過につれて産卵率は大となつた。

2. アラ液化物とアラ液化物の原料である生魚屑との効果について比較した。乾物として 2.5% のアラ液化物と生魚屑 10% を植物性飼料に混じて与えたものの産卵率は，生魚屑 15% を与えたもののそれより常に優れていた。

3. 乾物として 2.5% のアラ液化物を 9 ケ月間与えたところ，基本飼料のみを与えたものより生み疲れの時期に多少優れた結果を示した。

本研究は昭和28年度の文部省科学試験研究費で行つた。研究に協力された深見泰雄，楠原正徳，花岡潔，大庭正利，中西重雄の諸氏に深謝の意を表す。

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Biochemical Studies on the Liquefaction of Fish Body—Ⅲ. On Effectiveness of Fish-Autolysate to the Culture of Bacteria

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Biochemical Studies on the Liquefaction of Fish Body—III. On Effectiveness of Fish-Autolysate to the Culture of Bacteria

Tetsuo TOMIYAMA and Azuma KAWAZOE

It has been an urgent problem in Japan to prepare a substitute for LIEBIG meat extract by making use of fishery products, since we have a poor resource of live stocks. Many studies have been carried out with hopeful results.

As early as in 1900, it has been shown by TOZUKA[1] that a number of bacteria were able to grow normally on a hot watery extract of either fresh fish or its dried product to a similar extent on the meat extract. Any difference in growth rate of bacteria has not been observed between squid extract and Liebig extract[2]. An extract of oyster[3] or Laminaria[4] has also been found to be comparable to the meat extract. HIDAKA[5] and ARIZAWA[6] have reported that a good growth response of bacteria similar to that on Liebig extract was obtained on a hot watery extract of bonito which is a by-product in making the dried bonito. TAKEBAYASHI[7], however, has indicated that it is inadequate for the preservation of strain or isolation of new species without the addition of an alcoholic extract of the rice bran. ITOYO[8] has shown that an extract of fish, if used instead of peptone, was found to be an appropriate culture medium for *Vibrio cholerae*. KIMATA[9] has obserbed that a hot watery extract of fish meal could also be used without any inferior result than LIEBIG extract. ARIMA[10], and TOYAMA and YASUKAWA[11] have carried out detailed experiments showing that a warm watery extract of sardine prepared according to OGURA's procedure gave a little better rerult than LIEBIG extract in the rate of growth and indol- and toxin-formation of several pathogenic bacteria. A similar result has been obtained by a hot watery extract of sardine[12]. TSUKAHARA[13] has reported that a digest of whale meat by pepsin was more effective than LIEBIG extract, whereas a hydrolysate of the whale meat with 20 per cent hydrochloric acid was less effective.

It has intensively been studied in our laboratory during World war II to prepare an effective culture media by the use of fishes as its material. A success was finally made in getting a mixture of peptone and amino acids in an industrial scale by subjecting whole fish body to autolysis under a certain condition[14]. It has been found that the preparation is very rich in ingredients which promote the growth of bacteria. It is quite a striking fact that just a simple solution of fish-autolysate without add-

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ing anything else is very effective to the culture of *Corynebacterium diphtheriae* as well as *Streptococcus hemolyticus* which are not oridnarily able to grow on the usual culture media. The chemical nature of the growth factor of the preparation is under investigation.

Before going further the authors wish to express their thanks to Prof. T. Toda of the bacteriological laboratory in the Faculty of Medicine of the Kyushu University for his hearty cooperation during the work. Thanks are also extended for a grant from the Research Fund of the Ministry of Education.

I. Method and Materials for the Experiment

1. The preparation of fish-autolysate and its composition

The fish-autolysate was obtained by subjecting whole fish body to the autolysis at about 50°C. and in slightly acidic reaction[14]. The liquefied part after being neutralized was clarified and concentrated to a syrup and then dried in a desiccator under reduced pressure. The powdered preparation has the following composition on average: moisture, 5 per cent; nitrogen, 13 per cent; ash, 9 per cent. The fractionation of various forms of the nitrogen according to Wasteneys and Borsook's procedure[15] gave, on average, values shown in Table 1.

Table 1. The analytical result of the fish-autolysate.

	Fish-autolysate		A commercial peptone	
	Per cent Total nitrogen	Per cent dry matter	Per cent total nitrogen	Per cent dry matter
Protein- and metaprotein-N	1.91		1.21	
Proteose-N	11.80		31.38	
Peptone-N	20.39		6.18	
Subpeptone-N	26.00		} 61.23†	
Amino acid-N	39.90			
Total nitrogen		12.98		12.74*
Ammonia		0.365		
Creatinine		2.12		
Ash		13.82		
Calcium		2.88		
Magnesium		0.089		
Chloride		7.75		

* The nitrogen contents of polypeptone (Takeda Co.), Teruuchi peptone and a peptone supplied by Katayama Co. were 12.7%, 12.0% and 12.1%, respectively.

† Sum of subpeptone-N and amino acid-N.

2. The experimental method

Several species of bacteria mainly pathogenic were used for the experiment. A simple two per cent solution of fish-autolysate was used for the preparation of the liquid media and the agar plate media. As control media two kinds of the usual media were used, namely, one containing one gram of the extractive of bonito and one gram of the Teruuchi-peptone per 100 cc., and the other containing one gram

of the LIEBIG extract and one gram of the Teruuchi-peptone per 100 cc. It has been noticed that, while egg white is ordinarily needed to clarify the agar media, the present preparation can be used without any process of clarification.

The comparison between the present preparation and the usual media was made, on one hand, by the turbidity as well as the intensity of the indol reaction in case of culture in liquid media, and, on the other, by an outer appearance of colonies developed on the agar plate media.

II. Experimental Results

1. The growth in liquid media and on plate media

The results are shown in Tables 2 and 3. From these findings it will be clear that the present preparation is superior to the control media. It has also been noticed that *B. pyocyaneus* cultured by the fish-autolysate forms pyocyanin in a remarkable quantity not comparable to the control media. Furthermore, the color reaction due to indol was observed to be much more distinguishable than that of the control media,

Table 2. The growth of several bacteria in liquid media observed after a 24-hour incubation at 37°.

Species of bacteria	Kinds of culture media*	Turbidity	Indol reaction	
			KITASATO-SALKOWSKI	PRINGSHEIM
E. coli	(a)	++	±	++
	(b)	++	−	+
	(c)	++	−	+
Staphyl. aureus	(a)	++	−	+
	(b)	++	−	+
	(c)	++	−	+
Bac. proteus	(a)	++	−	+
	(b)	+	−	+
	(c)	+	−	+
Bac. subtilis	(a)	++	−	−
	(b)	+	−	−
	(c)	++	−	−
Bac. pyocyaneus	(a)	++		
	(b)	++		
	(c)	++		
Corynebacterium diphtheriae	(a)	++		
	(b)	−		
	(c)	−		
	(d)	−		
	(e)	−		
	(f)	−		
	(g)	−		
	(h)	−		

* Per 100 cc. solution, media (a) contains 1 g. of the fish-autolysate and 1 g. of the Teruuchi peptone ; media (b) contains 1 g. of a bonito-extract and 1 g. of the peptone ; media (c) contains 1 g. of the LIEBIG extract and 1 g. of the peptone ; media (d) contains 2 g. of the LIEBIG extract ; media (e) contains 1 g. of a fish-extract prepared according to OGURA's procedure and 1 g. of the peptone ; media (f) contains 2 g. of the fish-extract ; media (g) contains 2 g. of the peptone ; media (h) contains 2 g. of a peptone supplied by Katayama Co. The concentrations of these ingredients are all based on dry basis. All these media contain sodium chloride at a 0.3 per cent level.

Table 3. The growth of severbl bacteria on plate media observed after a 24—hour incubation at 37°

Species of bacteria	Colonies				Order of superiority	Species of bacteria	Colonies				Order of superiority
	Kinds of culture media*	Abundance	Largeness	Thickness			Kinds of culture media*	Abundance	Largeness	Thickness	
E. coli	(a)	###		##	1	*Vibrio cholerae*	(a)	##	###		1
	(b)	##		+	2		(b)	+	+		3
	(c)	+		+	3		(c)	+	##		2
	(d)	+	no difference				(d)	+		no difference	
	(e)	±					(e)	##			
	(f)	±					(f)	+			
	(g)	±					(g)	+			
	(h)	+					(h)	##			
Staphyl. aureus	(a)	##	###	###	1	*S. paratyphi* A	(a)	###	###	no difference	1
	(b)	##	##	###	2		(b)	+	+		3
	(c)	##	##	##	3		(c)	##	##		2
	(d)	##				*S. paratyphi* B	(a)	###	##	###	1
	(e)	+					(b)	+	+	+	3
	(f)	##					(c)	##	##	##	2
	(g)	+									
	(h)	##					(a)	###	##	###	1
Bac. proteus	(a)	##	no difference	###	1	*Bac. typhi*	(b)	+	+	##	3
	(b)	+		##	2		(c)	+	##	+	2
	(c)	+		+	3		(d)	+			
Bac. subtilis	(a)	###	no difference	no difference			(e)	##			
	(b)	###					(f)	+			
	(c)	###					(g)	+			
							(h)	##			
Bac. pyocyaneus	(a)	##	###	+	1	*Bac. dysenteriae*	(a)	##	###	no difference	1
	(b)	+	##	##	3		(b)	##	+		3
	(c)	+	##	###	2		(c)	##	##		2
Strept. haemolyticus	(a)	###			1		(d)	±			
	(c)	—			2		(e)	+			
							(f)	±			
							(g)	±			
							(h)	±			

* Notations of media are the same as those given in Table 2.

All these media contain agar—agar at a 3 per cent level.

probably being due to its high tryptophan content (1.0—1.3 per cent) and also to a pale coloration of liquid media.

2. **some observations in culturing certain pathogenic bacteria**

a) The rate of growth of *Vibrio cholerae.*

When 5 cc. of two per cent fish-autolysate were innoculated with *Vibrio cholerae* in an amount as minute as 1/100,000 a platinum-loopful quantity of the bacteria, a distinct turbidity and the cholera rose reaction appeared after a 5- to 6-hour incubation at 37°C. In the control experiment using LIEBIG extract, however, the reaction was positive only after a 24-hour incubation. This result indicates that an early diagnosis of cholera disease will be realized without any difficulty by use of the present preparation.

b) The culture of *Corynebacterium diphtheriae.*

The rate of growth of *C. diphtheriae* which was observed in the liquid media of the present preparation was two to three times greater than that in LOFFLER's or MARTIN's media ; a 24-hour incubation after innoculating to two per cent solution of the present preparation was enough to obtain a fairly good formation of the membrane on the surface of the liquid media.

The toxin which was obtained after being cultured through two successive generations by two per cent solution of the present preparation showed M. R. D. of 0.000001 cc. on being tested by RÖMER's method.

The volutin of the bacteria grown on the plate media can clearly be observed without fail after staining. Therefore, by the use of the preparation, it will become to be quite feasible to identify *C. diphtheriae* and to efficiently manufacture the anatoxin.

3. **The change of growth-promoting activity during autolysis**

From the point of view to prepare an efficient culture medium and further to elucidate the chemical nature of the growth-promoting ingredient, it will be of interest to see the change in the growth rate in relation to the autolytic degradation of fish body. The agar plate media which contained at a two per cent level the autoly-

Table 4. The change in growth-promoting activity with progress of autolysis.

Species of bacteria	Time of incubation in hour	Time of autolysis in hours											
		1	1.5	2	3	5	8	13	23	28	33	42	68
E. coli	24	++	++	++	+++	+++	+++	+++	+++	+++	++	++	+
	48	++	++	++	+++	+++	+++	+++	+++	+++	+++	++	+
Staphyl. aureus	24	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
	48	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Bac. dysenteriae	24	+	+	+	+	++	++	+++	+++	++	++	+	+
	48	+	+	+	++	+++	+++	?	+++	+++	++	+	+
Bac. typhi	24	++	++	++	++	+++	+++	+++	+++	+++	++	++	+
	48	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Vibrio cholerae	24	++	++	++	++	++	++	++	++	++	++	++	++
	48	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Coryn. diphtheriae	24	+	++	++	++	++	+++	+++	+++	+++	+++	+++	++
	48	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++

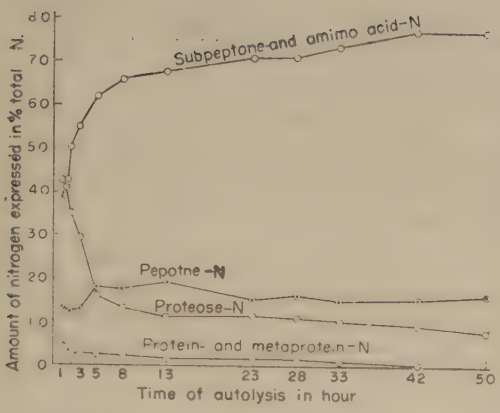

Fig. 1. Change of molecular size of nitrogenous compounds with the progress of autolysis.

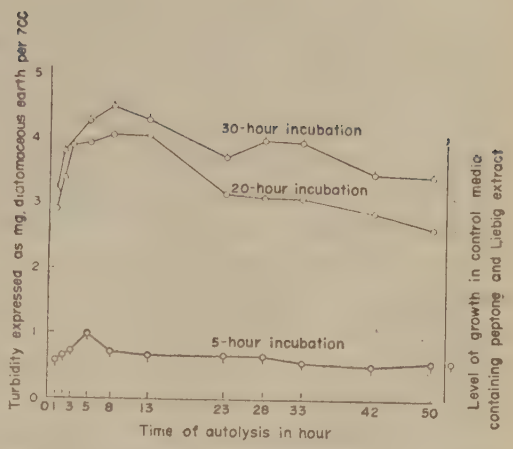

Fig. 2. Rate of growth of *E. coli* in the autolysates having varying degree of autolysis.

sates of sardine having different degrees of cleavage were innoculated with several kinds of bacteria. The qualitative differences in growth of the bacteria among these media are shown in Table 4. On the other hand, the changes of molecular size of nitrogenous compounds with the progress of the autolysis are shown in Fig. 1. It will clearly be seen that the growth-promoting activity shown by the autolysates varies in general with the progress of the antolysis. Such a difference in the activity, however, was hardly observed for *Staphylococcus aureus* and *Vibrio cholerae*.

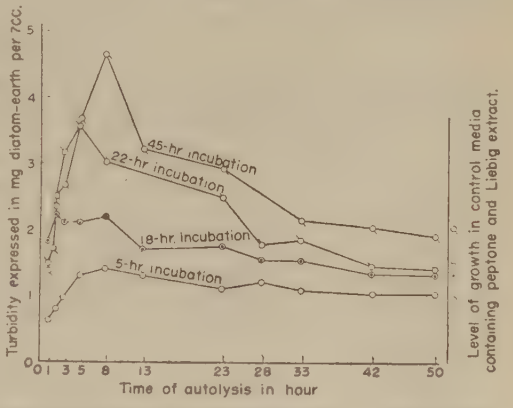

Fig. 3 Rate of growth of *Bac. typhi* in the autolysates having varying degree of autolysis.

The quantitative determination has been made of the growth rates of *Bac. typhi* and *E. coli* in the liquid media by comparing the turbidity due to the bacteria against an arbitrary standard made of a suspension of diatomaceous earth. Figs. 2 and 3 show that the autolysate obtained after 5~13 hour autolysis gave the maximum rate of growth for these two kinds of bacteria. This maximum activity appears to occur after the marked decrease in the proteose fraction. It needs a further study, however, to determine whether the peak in growth rate is due to that the growth factor might be available in maximum quantity or due to that the forms of nitrogen be suitable to the bacterial growth.

III. Summary

1) A preparation which is effective in culturing bacteria instead of LIEBIG extract and peptone has been obtained by autolyzing whole fish body.

2) Experiments have shown that it is superior to the usual media when judged from the magnitude of colonies on the plate media and the turbidity of the liquid media

3) The liquid media gave a distinct indol reaction on culturing either *E. coli*, *Staphyl. aureus* or *Bac. proteus*. The liquid media gave the easily perceptible cholera rose reaction after a 6-hour incubation at 37°C. after innoculating *Vibrio cholerae* in a quantity as minute as 1/100,000 a loopful bacteria.

4) *Corynebacterium diphtheriae* was able to grow on a simple two per cent solution of the present preparation with a growth rate two to three times greater than on MARTIN or LOFFLER's media. The volutin of the bacteria grown on the preparation could be identified without fail on staining. The minimum skin reaction dosis of the toxin formed was 0.000001 cc.

5) It has been shown that the activity of promoting the growth of bacteria which was displayed by the preparation was in variance with the progress of the autolysis.

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肝臓の未知動物蛋白因子に関する研究—I.
ピリジン毒に対する肝中の未知APFの効果*

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Studies on Unidentified APF in Liver—I.
Alleviative Effect of a Purified Fraction of Liver on the Toxicity of Pyridine

Tetuo Tomiyama and Yasuo Yone

It has been reported by Dinning[1] that the addition of vitamin B_{12} to a pyridine-containing diet improved the growth of the rats. In a previous paper[8] authors showed that the chick growth promoting effect of bovine- and bonito-liver is due to a new chick growth factor which is nearly free from vitamin B_{12} and folic acid. Therefore, we studied on the effect of the unidentified vitamin concentrate to alleviate the toxicity of pyridine. The results obtained can be summarized as follows.

1. Weekly intramuscular adminstration of the concentrate at 232 mg level which was equivalent to 17.7 g of the fresh bovine liver, improved the growth of albino rats fed a pyridine(1.5%)-diet for 12 days prior to the experiment.

2. An alleviative effect was observed when vitamin B_{12} was administered intramuscularly weekly at a 3 microgram level.

3. When a purified concentrate of the unidentified vitamin at a level equivalent to 30 g of fresh bonito liver was intramuscularly administered weekly to rats fed the pyridine (1%)-diet for a 18-day period prior to the start of the experiment, the survival time was prolonged from 4.2 days in the control to 16.5 days, and a half of the rats in the experimental group survived even after the end of the experiment, i. e., 21 days.

I 緒 言

ピリジン摂取による白鼠の発育阻害は肝抽出物或はビタミン B_{12} の投与によつて防止されることがDinning氏等[1]により報告されている。他方著者等[2)~7)]は肝抽出物にはフェニールヒドラヂン及びブロミンによる貧血抑制成分があり、ブロミン貧血抑制成分はビタミン B_{12} 及び葉酸とは異なる未知成分であること、また高窒素耐性能を強化する有効成分の存在することを報告した。他方また牛及びカツオ肝中の鶏雛生長促進効果は主として B_{12} 以外の新しい因子"ビタミン B_{15}"[8]によることを明かにした。依つて本研究はピリジン毒に及ぼすビタミン B_{15} 濃縮物の効果について検討した。

II 実 験 方 法

1. 試 料

ビタミン B_{15} 濃縮物及び精製物：牛或はカツオの肝臓に適量の湯及び塩酸を加えて pH 4.5 とし、50~55 °C で数時間放置した後に濾過し、濾液（肝液化物）を分取した。之に活性炭を加えて攪拌 30 分後に濾過し、活性炭を水で洗い、洗液は非吸着液と合わせた。活性炭に吸着された B_{15} 濃縮物は苛性ソーダ稀溶液（pH 9）で溶出した。また B_{15} 濃縮物を再び上記のように活性炭処理し B_{15} 精製物を得た。各試料は中和後，減圧濃縮して実験に供した。

1956 年 5 月 25 日受理
* 本研究の大要は昭和 27 年 9 月函館に於ける日本水産学会大会に於いて発表した。

ビタミン B_{12}：武田薬品工業K・K製“フレスミン”注射液（1 cc=15 μg ビタミン B_{12}）を用いた。

2. B_{15} 濃縮物の効果

1952 年5月 22 日及び 25 日に生れた2腹の合仔7尾を供試した。これらの白鼠は体内のＡＰＦを消耗させるため，Table 1 の脚註に示したピリジンを1.5％添加した飼料（ピリジン1.5％飼料と略称）のみで予め 1952 年6月 25 日より7月6日まで飼育した。7月7日に4区に分け，B_{15} 区には生牛肝 17.7g より得た乾物として 232 mg の B_{15} 濃縮物を，B_{12} 区には 3 μg の B_{12} を，また B_{12}+B_{15} 区には 3 μg の B_{12} と生牛肝 17.7 g より得た B_{15} 濃縮物を毎週3回に分けて筋肉注射し，7月 27 日まで飼育した。

3. B_{15} 精製物の効果

1953 年5月 18 日に生まれた2腹の白鼠仔 18 尾を用い，1953 年6月 21 日より7月8日まで Table 2 の脚註に示したピリジンを1％添加した飼料を与え，7月9日迄生存した 10 尾を3区に分けた。7月9日以降は対照（A）区及び試験区にはピリジン添加飼料の給与を続行した。対照（B）区はピリジンの投与のみを中止した。また試験区には生カツオ肝 30 g より得た B_{15} 精製物を1週3回に分けて毎週筋肉注射し8月 30 日まで飼育した。いずれの試験も給水及び給餌は自由攝取とした。

III 結 果 及 び 考 察

1. B_{15} 濃縮物の効果

供試鼠は代々普通繁殖飼料で飼育した鼠から生まれた仔であるから，体内にＡＰＦを多量に蓄積しているだろう。依つて本研究では体内に蓄積しているＡＰＦを消耗させるために予め 12 日間ピリジン添加飼料で飼育した。生長試験の結果は Table 1 に示す通りで，B_{15} 濃縮物区，B_{12} 区及び B_{15} 濃縮物・B_{12} 併用区の生長は対照区の生長を凌駕し，約2倍の生長を示した。

Table 1. Growth response of pyridine-fed albino rat to vitamin B_{12} and vitamin B_{15} concentrate from bovine liver. (July 7 '52～27)

Supplements	Weekly dose of supplements. Intramuscular administration. Dry basis.	Albino rat No.	Body weight in gm. Before 12 days pyridine-feeding	Initial (July 7)	After 10 days	After 20 days	Gain in
None		501	40	66	75	79	13
		507	37	62	73	73	11
B_{15} concentrate*	232 mg	502	37	52	66	76	24
		509	34	60	58	80	20
Vitamin B_{12}**	3 μg	504	33	60	70	83	23
V. B_{12}+ B_{15} concent.	3 μg 232 mg	506	35	50	68	83	33
		510	37	61	68	79	18

* The solubilized liver was treated at pH 4.5 with activated charcoal, followed with the elution with a dilute sodium hydroxide olution at pH 9. On dry basis crude protein, 35.2％; NaCl, 33.9％. ** Vitamin B_{12} “Fresmin” 1cc=15 μg V. B_{12}

Rats were fed a basal pyridine-ration for 12 days before the experiment. The basal pyridine-ration was composed of sucrose 58, soybean meal 30, yeast 3, McCollum salt 2, vegetable oil 5, vitamin A 500 I.U and pyridine 1.5.

2. B_{15} 精製物の効果

この試験では予め 18 日間ピリジン添加飼料で飼育し，体内に蓄積したＡＰＦを消耗させた白鼠を用いた。また本試験ではレシチン及び各種の必須ビタミンを基本飼料に添加して B_{15} 精製物の効果をみた。試験の結果は Table 2 に示す通りで，ピリジン添加飼料のみを与えた期間が永かつたためか，対照（A）区の大部分が試験開始後3日内に斃死したので，生長では比較できなかつた。なおピリジン投与を中止した対照（B）区は正常生長に恢復した。他方生存日数を比較すると対照（A）区が試験開始後1～11日内平均 4.2 日目に斃死したのに比べて，B_{15} 精製物区は4尾の内2尾が試験期間中生存し，平均 16.5 日生存した。Dinning 氏等の研究を引用すれば，以上の結果は明かに B_{15} 区分が攝取したピリジンのメチール化に関与していることを示唆している。

Table 2. Effect of vitamin B_{15} concentrate on survival of pyridine-fed albino rat.

Albino rat No.	Sex	Supplements	Weekly dose of supplements. Intramuscular administration. Dry basis	Body weight in gm. Before 18 days	Initial	After 1wk.	After 2wks.	After 3wks.	Days of survival
103	♂	None* (A)		30	45	—	—	—	1
106	♀			34	74	—	—	—	3
110	♂			36	78	—	—	—	3
113	♀			39	63	86	—	—	11
18	♂			33	68	—	—	—	3
102	♀	None**(B)		34	67	98	113	129	21
101	♂	B_{15} §	270 mg	28	58	93	—	—	8
104	♀			34	53	50	52	55	21
109	♂			34	71	74	91	103	21
116	♀			33	69	81	109	—	16

* Basal diet used was composed of sucrose 49, soybean meal 40, yeast 5, McCollum salt 3, olive oil 3, pyridine 1, niacin 3 mg, Ca-pantothenate 1 mg, V.A 520 I.U., pyridoxine-HCl 0.3 mg, biotin 10 μg, B_1-HCl 0.4 mg, B_2 0.2 mg, folic acid 0.4 mg, V.K_3 0.1 mg, lecithine 1 g per cent diet. ** Pyridine was omitted from the basal diet after 18 day preliminary feeding of the pyridine-diet. § This was obtained by repeating twice the procedure of adsorption by charcoal and elution with dilute sodium hydroxide solution.

IV 総　　括

1. 予め 12 日間ピリジン添加飼料を与えた白鼠に生牛肝 17.7 g より得たビタミン B_{15} 濃縮物を毎週筋肉注射したところ，ピリジンの生長阻止作用を軽減した。

2. 毎週 3 γ/g のビタミン B_{12} 注射はピリジン毒作用を軽減した。

3. 予め 18 日間ピリジン飼料を与えた白鼠に，毎週生カツオ肝 30 g より得た B_{15} 精製物を注射したところ，斃死日数は対照の 4.2 日に対し 16.5 日を示した。また対照が 11 日内に全て斃死したのに対し，試験区は半数が 21 日の試験期間中生存した。

本研究は昭和 27 年度の文部省科学研究費で支弁した。なお動物の飼育に尽力された土井良平次郎氏に深謝の意を表わす。

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Absorption of Dissolved ^{45}Ca by Carassius auratus

By

Tetuo Tomiyama Shinya Ishio and Kunio Kobayashi

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Tetuo Tomiyama*, Shinya Ishio** and Kunio Kobayashi***

1. Introduction

Several works[1)] have already shown that the fresh water fish can most probably absorb calcium ion from the surrounding water. No works have been made, however, of a probable pathway for calcium whether orally or from the surface. The present paper deals with the elucidation of this pathway which is essential to studies on the contamination of fishes with the fission products and on their decontamination.

2. Experimental Method and Results

The sample was placed in a sample dish of stainless steel, dried, charred by the burner and reduced to ash in the electric muffle furnace at about 700°C. The radioactivity was measured at 1 cm distance from the G-M Counter with Scaler Model 16 of the Scientific Research Institute, Tokyo. Correction for the self-absorption was made for each sample depending on the weight of its ash.

i) Absorption and accumulation of ^{45}Ca by *Carassius auratus*

Ten gold fishes ranging in body weight from 2 to 3.5 gr were placed in a vat ($36\times28\times6\,cm^3$) containing three liters of a well-water supplemented with ^{45}Ca in the form of calcium chloride and adjusted to pH 7. This radioactive water contained calcium of 111 mg/L and the radioactivity of 2,450 cpm/ml. During the experiment, the temperature ranged from 7 to 9°C. and no feed was supplied. After appropriate intervals the fish was taken up, let it swim in a streaming water for ten minutes to remove a surface contamination, and then sacrificed by destroying its nervous centre. The whole body was dried, incinerated in a crucible, transferred to the sample dish, and subjected to the radioactivity measurement. Fig. 1 illustrates that the rate of deposition of ^{45}Ca was fairly remarkable and that this rate was varied with the activity of the fish.

In order to see the distribution of ^{45}Ca in various parts of the body, a similar experiment was carried out using the fish of 19 to 29 gr in body weight. The fish was sacrificed at intervals, and the various parts were removed. After weighing, the samples were dried, incinerated, and subjected to the radioactivity measurement. As is shown in Fig. 2, a marked rate of ^{45}Ca deposition was observed in the gill,

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*** Research-fellow, Ditto

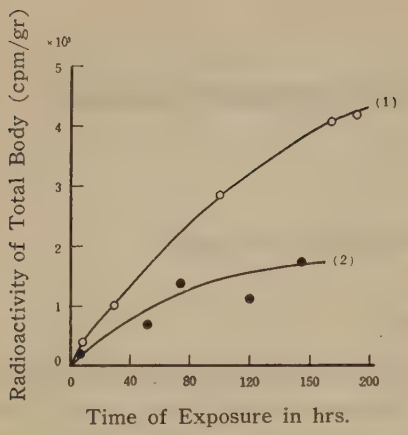

Fig. 1. Rate of increase of total radioactivity in *Carassius auratus* (Linne) following its exposure to ^{45}Ca.

Curve 1 shows the rate in a group of fishes which showed active movement at about 9°C; Curve 2, the rate in another group of fishes which were remained sluggish at about 9°C.

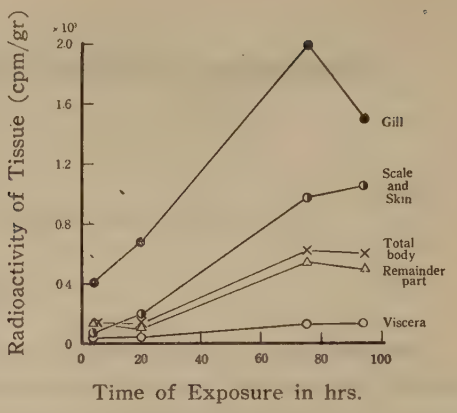

Fig. 2. Absorption of ^{45}Ca ion by *Carassius auratus* (Linne)

● Gill, ◑ Scale and Skin, × Total body, △ Remainder part, ○ Viscera.

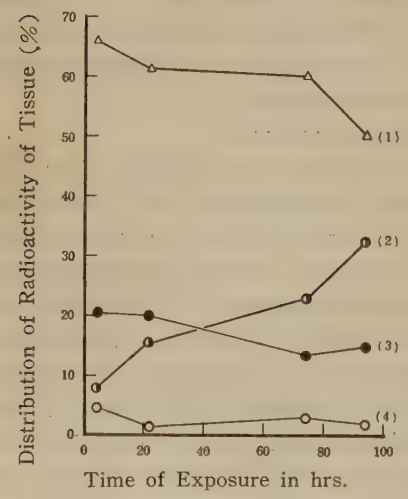

Fig. 3. Variation of distribution of absorbed ^{45}Ca with time of exposure of *Carassius auratus* (Linne).

Curve (1), body except scale, skin, gill, and viscera; (2) scale and skin; (3) gill; (4) viscera.

next in the scale, and the least in the viscera. The radioactivities of various parts expressed as per cent of that of total body at each interval are shown in Fig. 3. It is to be noted that the value for the gill decreased more or less with time in contrast to that for the scale which increased remarkably with time of the exposure.

ii) Pathway for absorption of ^{45}Ca ion

The foregoing experimental results suggest that ^{45}Ca might not be absorbed orally but absorbed either through the skin or the gill. It is essential, however, in order to find out the absorption channel for ^{45}Ca that the gill shall be separated as much as possible from the other surface of the body. An apparatus shown in Fig. 4 has been devised to meet this requirement. The fish was bound at just a hinder part of the opercle with a thin rubber collar, edge of which was fixed with a glass joint shown in Fig. 4. The whole apparatus was placed in an aquarium. In this way, it becomes possible to expose only the head part (B) to ^{45}Ca ion without contaminating the remainder part

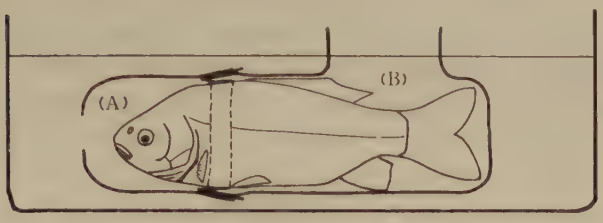

Fig. 4. Apparatus for determination of absorption channel of ^{45}Ca dissolved in water.

(A), or vice versa.

First, the head part was exposed at 9°C. to a water containing 9,300 cpm/ml and 125 mg calcium per L. A constant bubbling of oxygen was made during the experiment. The other chamber was filled with a well-water without ^{45}Ca ion. After 18-hour exposure, the radioactive water was first drained, the fish was taken out, and its surface contamination was removed as described above. Then, another experiment was carried out in which the remainder part was exposed to a water containing 27,000 cpm/ml and 260 mg calcium per L., while the head part was streamed with a well aerated water of 15°C. The fish after being subjected to this fixed condition for 21 hours, was treated in the same manner as in the former experiment. Though the conditions of exposure in these experiment were not alike, data obtained are summarized for the convenience of comparison in Tables 1 and 2. It is clearly

Table 1. Total radioactivity, cpm in tissues of *Carassius auratus* (Linne).

Site of absorption \ Tissue	Gill	Scale	Skin	Viscera	Vertebra	Remainder part	Total body	Radio-activity of water	Body weight
(1) Surface except head	107	1,263	13	8	10	2,570	3,971	cpm/ml (A) 27,000	gr 24.05
(2) Head with gill	5,785	3,002	73	365	519	14,250	23,994	(B) 9,300	22.65
Ratio of (2) to (1)	54	2.4	6	46	52	5	6		

Table 2. Radioactivity, cpm per gr of fresh tissue of *Carassius auratus* (Linne).

Site of absorption \ Tissue	Gill	Scale	Skin	Viscera	Vertebra	Remainder part	Total body
(1) Surface except head	92	996	13	3	22	145	165
(2) Head with gill	5,284	2,480	76	114	1,067	874	1,063
Ratio of (2) to (1)	59	2.5	6	48	49	6	6.4

seen that exposure of the gill to ^{45}Ca ion gave rise to a great deposition of ^{45}Ca in the gill, scale and vertebra, and to a much greater deposition than without exposing the gill, notwithstanding in exposing the gill to ^{45}Ca the condition was less amount of ^{45}Ca in the water, lower temperature, and shorter duration of the exposure.

3. Discussion

The dynamic aspects on the metabolic pathway of radiocalcium has been studied by several investigators[1]. Almost no works, however, have been reported of the metabolism of radiocalcium in fishes. On the other hand, it has long been known that fishes are most likely able to absorb calcium from the environment. Sekine[2] reported that an appreciable increase of calcium content of salmon yolk-sac fry was noticed after keeping it for 38 days without feeding. McCay et al[3] obtained analytical data showing that trout fry fed only liver for ninety days accumulated calcium in an amount much greater than that supplied by liver. Kaplanski and Baldirewa[4] demonstrated that keeping the carp for about two months in a tap water containing 2 per cent calcium chloride, resulted in an increase of calcium in the muscle. It has further been observed by Usui et al[5] that *Hypomesus olidus* living in brackish water contained calcium in an amount larger than that in fresh water. Tomiyama and Kojima[6] made it clear that mortality of young trout was inversely proportional to the contents of calcium in its living water. These results have been evidenced by the present experiment which showed the rate of uptake of ^{45}Ca from the water to be appreciable in the gold fish.

It has hitherto remained unsolved what part of the fish body is responsible for the absorption of calcium. Possibilities are three alternatives for the absorption channel of calcium; the one is the digestive tract, the second, the skin, and the third, the gill. The presence of only a small amount of ^{45}Ca in the viscera as is shown in Fig. 1 suggests that the uptake may not primarily occur through the digestive tract. It is also conceivable from the point of osmo-regulation that the fresh water fish will not drink water in contrast to the marine fish[7]. Data given in Tables 1 and 2 also infer that the absorption of calcium through the digestive tract may take place to the least extent even if really occurred. Then, it will be presumed that the absorption will take place from a certain site or all over the surface of the body. By using the technique described above, it has been indicated that a pathway for ^{45}Ca is located on the head part. That a main pathway exists nowhere but the gill is almost no doubt from data given in Table 1 showing an enormous increase of ^{45}Ca in the gill. Judging from data presented in Fig. 2, a translocation of ^{45}Ca from the gill to the scale appeared to take place, since a gradual rise in the activity of the scale was observed as compared to an abrupt rise in that of the gill. This will be clearly seen by expressing the extent of deposition of ^{45}Ca as per cent of the radioactivity of the total body. This value for the gill was decreased whereas that for the scale increased with time of exposure to ^{45}Ca ion.

On the other hand, the presence of ^{45}Ca at very low level in the digestive tract

will not necessarily mean that the water might never have been drunk, if the rate of absorption of ^{45}Ca in the digestive tract would be rather great. Furthermore, a very high level of ^{45}Ca in the gill may also be attributed to adsorption of ^{45}Ca on the gill. Therefore, for the conclusive determination that the gill is really a main pathway for calcium absorption will be needed the estimation of rate of increase of ^{45}Ca in the blood besides the gill, and also removal of the absorption channel through the digestive tract by such an appropriate technique as ligature or impregnation. These requisites were realized in a study on the absorption of ^{90}Sr by carp which will be dealt with in a later report.

4. Summary

1. The rate of the absorption and deposition pattern of ^{45}Ca in *Carassius auratus* have been determined.

2. When the fish was kept in a well-water containing ^{45}Ca ion, the radioactivity was increased remarkably in the gill as well as the scale, but the least in the viscera.

3. A technique has been devised for segregating the head part including the gill from the remainder part and for a separate exposure of each part to ^{45}Ca ion.

4. It has been clearly demonstrated that when only the head part was exposed to ^{45}Ca ion, an enormous deposition of it took place as compared to exposing only the remainder part to ^{45}Ca ion.

5. It has been postulated that the gill is most likely a main pathway for ^{45}Ca ion.

In conclusion, thanks are extended for a grant from the Research Fund of the Ministry of Education.

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Absorption by Carassius auratus of ^{45}Ca contained in Rhizodrilus limasus

By

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Tetuo Tomiyama*, Shinya Ishio** and Kunio Kobayashi***

1. Introduction

The present authors[1] have reported evidences that Carassius auratus absorbed ^{45}Ca ion primarily through the gill. It is to be supposed that the supply of calcium from the feed might be insignificant for its metabolic requirement. It will be of interest, accordingly, to compare the absorption rate of ^{45}Ca from the feed by the gold fish as well as the albino rat, and also to estimate in the gold fish the absorption rate through the intestine as compared with that through the gill. The present study has been undertaken to answer these problems and thus to throw some light on the route of the comtamination of the fish with the fission products.

2. Experimental Method and Materials

A preliminary experiment showed that it required a 10-hr exposure to reach an equilibrium between the ^{45}Ca concentrations outside and inside of a worm, *Rhizodrilus limasus*, which is frequently found in the bottom-muds of the domestic sewage. So, the following procedure was employed to obtain the worm rich in ^{45}Ca.

About 25 gr of the worm were kept for three days in 1.7 *l* of a well-water which was adjusted to pH 7 following the supplementation with 1.7 cc of $^{45}Ca\ Cl_2$ having 0.19 mc/cc and 95.2 mg calicium per cc. This well-water possessed the radioactivity of 9,300 cpm/cc and the calcium content of 125 mg/*l*. So, the radioactivity of 74.4 cpm was equivalent to one microgram of calcium. The worm thus treated was found to contain 147 microgram of calcium and 11,000 cpm/gr of radioactivity fresh matter, i. e., 74.8 cpm/gr being equivalent to one microgram of calcium. Since this ratio of calcium to the radioactivity for the worm was in good agreement with that for the radioactive water employed, it is apparent that a complete equilibrium was established between outside and inside of the worm.

In order to see the absorption rate following the ingestion of the radioactive worm, it is prerequisite to use a fish which can ingest the worm within a short time-interval, since otherwise the radiocalucium content of the worm will change with time depending on that of the water. It was observed that *Carassius auratus*

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was best suitable to this type of experiment. Eight gold fishes ranging in body weight from 3 to 5 gr were kept for a week in 2.5 liter of a well-water which had been conditioned for calcium content by adjusting to a concentration of the sample radioactive water, i. e., 76.8 mg/*l*. Thus, there will be no danger to occur the change in the osmotic pressure as well as the dynamic equilibrium of calcium following the exposure to the radioactive water. Then, four fishes were transferred each to 500 cc of the calcium-conditioned non-radioactive water, and the other four fishes each to 500 cc of the radioactive water which was prepared by adding to a 2.5 *l*. well-water 1.25 cc of the $^{45}Ca\ Cl_2$ solution and adjusted to pH 7. The radioactive water showed the activity of 7,800 cpm/cc and the calcium content of 76.8 mg/*l*. Each fish was fed every day 0.1 g of the radioactive worms which had been washed for one minute in a streaming water and removed of adhered water with filter paper. Only a few minutes were needed to ingest this total amount of the worm. Each fish was taken up 24, 48, 96 and 193 hours following the administration, followed by a 10-minute washing in a steaming water, and then sacrificed. Various parts were removed, weighed, placed in a dish of stainless steel, dried and incinerated at about 700°C. For the radioactivity measurement was employed the G-M Counter with Nuclear scaler model 166. The sample ash was placed at 1 cm distance from the mica window of the G-M Counter.

3. Experimental Results and Discussion

Table 1 and 2 show the distribution of ^{45}Ca as expressed in cpm per gr firesh tissue and in per cent of the radioactivity of the total body, respectively. It is

Table 1. ^{45}Ca of various tissues of gold fishes supplied either by water and feed or by feed only. Values given are expressed as cpm/gr fresh matter.

Absorption site / Exposure / Tissue	^{45}Ca supplied by water and feed				^{45}Ca supplied by feed only			
	Time of exposure (day)				Time of exposure (day)			
	1	2	4	8	1	2	4	8
Gill	—	13,552	21,303	49,617	—	61	210	370
Scale	—	29,196	47,896	101,351	—	224	654	1,285
Fin	—	21,105	27,248	54,008	—	185	273	621
Vertebra	—	12,439	22,879	56,243	—	73	199	441
Head	—	8,697	19,738	38,787	—	90	217	323
Skin	—	703	2,486	3,656	—	0	40	94
Eye	—	789	1,211	2,428	—	14	56	20
Muscle	—	377	848	1,745	—	21	14	23
Body except viscera	2,127	8,616	16,660	32,493	42	70	182	324
Digestive tract	—	7,764	7,650	10,333	—	615	1,200	2,252
Other viscera	—	309	597	883	—	7	6	5
Total viscera	2,092	3,462	3,224	4,547	367	223	319	615
Total body	2,119	7,522	13,744	25,972	105	102	211	396

noticed from Table 1 that the fish which was supplied with ^{45}Ca by both the water and the feed, deposited radiocalucium about one hundred times as much as the fish kept in the non-radioactive water. Consequently, the ^{45}Ca deposited in the various

Table 2. Distribution of radioactivity of fresh tissues in per cent that of total body.

Absorption site / Exposure / Tissue	^{45}Ca supplied by water and feed				^{45}Ca supplied by feed only			
	Time of exposure (day)				Time of exposure (day)			
	1	2	4	8	1	2	4	8
Gill	—	9.0	9.4	6.9	—	4.0	4.1	4.6
Scale	—	18.5	19.8	19.3	—	12.8	17.9	15.7
Fin	—	19.9	14.5	15.0	—	11.6	10.0	9.7
Vertebra	—	7.6	7.0	4.4	—	5.3	3.6	5.4
Head	—	16.2	25.2	29.5	—	9.5	15.1	13.0
The other bony tissue	—	17.7	16.7	19.1	—	9.8	14.3	10.9
Skin	—	0.4	1.2	0.5	—	0	1.0	0.9
Eye	—	0.2	0.2	0.2	—	0.3	0.6	0.1
Muscle	—	0.8	0.9	1.1	—	2.1	1.1	1.0
Body except viscera	79.5	90.2	94.9	95.9	32.6	54.3	67.5	61.3
Digestive tract	—	9.3	4.5	3.6	—	44.8	32.1	38.5
Total viscera	20.5	10.0	5.1	4.1	67.4	45.7	32.5	38.7

tissues, when supplied by both the water and the feed, can be estimated to derive largely from the radioactive water. Except that the scale was the highest in the radioactivity, the deposition pattern in this case was found to be similar to that given in the previous paper[1]. That is, the radioactivity was the highest in the scale and considerably high in the gill, fin, vertebra, skull and intestine in this order, and low in the viscera without the intestine, skin, eye and muscle. The high radioactivity in the intestine was contrasted to the previous data. This is partly due to ^{45}Ca of the feed which can be approximately estimated from the group with no supply of ^{45}Ca from the water. The other unaccounted-for activity in the intestine may be largely originated from ^{45}Ca excreted into the intestine, or that of the water ingested.

In the group with no exposure to the radioactive water, the radioactivity was the highest in the intestine. This appears to mean that ^{45}Ca of the worm remained there without appreciable absorption. Following the intestine, high value was in the scale and fin in this order. Moderately high value was recorded in the gill, vertebra and skull. The lowest value was in the muscle and eye. The high value in the gill may be either due to deposition of ^{45}Ca or to ^{45}Ca which was on the way to be excreted from the gill. Since no renewal of the water was made during this experiment, a question will arise as to the origin of ^{45}Ca deposited in the various tissues whether it came through the gill from some dissolved ^{45}Ca which had been excreted following the absorption of ^{45}Ca in the worm. Even if it is assumed that the total amount of ^{45}Ca of the worm be eliminated into the sur-

rounding water, only the radioactivity of 17.6 cpm/cc would have been appeared in the water even after eight days under the above condition. In addition, takeing into account a result, described elsewhere in this paper, that ^{45}Ca of the worm can be as much absorbed as only about 8-15 per cent, the above possiblity will not be worth consideration. At any rate, it is of interest that except the value for the intestinal tract, the deposition pattern of ^{45}Ca in this group was similar to that in the other group where ^{45}Ca was supplied from both the water and the feed.

In order to see how much calcium was deposited from the feed or the water, a calculation has been made using the following equation: the amount of deposited calcium from the worm=b/74.8 microgram; the amount of deposited calcium from the water=(a-b)/102 microgram, where a and b, the radioactivity, expressed as cpm per gram, of the gold fish kept in the radioactive water and the radioactivity of the fish kept in the non-radioactive water, respectively. Table 3 shows

Table 3. Amounts of absorbed Ca by gold fishes.
(Ca, γ per gram of fresh matter)

Time of exposure in days	Absorption of Ca contained in feed			Absorption of Ca dissolved in weter			Absorption rate of Ca feed : water	
	Absorbed Ca in total body	Absorbed Ca in body except viscera	Absorbed Ca per day	Absorbed Ca in total body	Absorbed Ca in body except viscera	Absorbed Ca per day	Of total body	Of body except viscera
	γ per gr	γ per gr	γ per gr. day	γ per gr	γ per gr	γ per gr day		
1	1.40	0.56	0.56	19.74	20.44	20.44	1 : 14	1 : 36
2	1.36	0.94	0.47	72.75	83.79	41.90	1 : 54	1 : 89
4	2.82	2.43	0.61	130.73	161.54	40.39	1 : 47	1 : 66
8	5.30	4.33	0.54	250.69	315.38	39.42	1 : 47	1 : 73

that the amount of calcium deposited in a group of the fish which had been exposed to ^{45}Ca ion and fed the radioactive worm, was sixty-six times as great as that in the other group of the fish only fed the radioactive worm. Althouth the amount of calcium administered orally was very small, the above data will be enough to conclude that the calcium in water can be overwhelmingly absorbed through the gill as compared with orally administered calcium. The assimilability by the gold fish of calcium in the worm has been calculated and given in Table 4. It will be seen from Table 4 that it amounted to only 8 to 15%. This low assimilability may be explained by a low rate of absorption of calcium from the intestine. According to Smith[2], this reasonning appears most probable. In connection to this low assimilability, it might be presumed that the calcium absorbed by the worm changed into an unabsorbable form. That this is not the case, however, can be shown in the following experiment.

A study has been made of the assimilability of calcium of the worm by the albino rat. A growing albino rat weighing 120 gr was fasted for 24 hours, fed two successive days each 5.5 gr of the same radioactive worm as was used for the gold fish, and sacrificed on the following day. The radioactivities of the gastro-intesti-

Table 4. Absorption rate of Ca contained in feed by gold fishes.

Time of exposure (days)	Total amounts of Ca in feed ingested	Amounts of absorbed Ca		Absorption rate of Ca	
		Body except viscera	Viscera	Body except viscera	Viscera
1	γ 14.7	γ 2.10	γ 4.33	% 14.3	% 29.5
2	29.4	2.45	2.06	8.3	7.0
4	58.8	6.62	3.18	11.3	5.4
8	117.6	9.76	6.16	8.3	5.3

nal tract, whole body without the gastro-intestine and the excretion were measured in the same way as above-described. Table 5 shows that the assimilability of

Table 5. Absorption by rat of Ca contained in *Rhizodrilus* fed.

	Rhizodrilus fed	Body except gastro-intestinal tract	Gastro-intestinal tract	Excrement
Total radioactivity (cpm)	120,450	95,750	15,800	5,400
Total amounts of Ca (γ)	1,610	1,280	211	72
Absorption rate (%)	100.0	79.5	13.1	4.5

calcium of the worm by the rat amounted to 79.5 per cent. It will be obvious, consequently, that the calcium absorbed by the worm can not be considered to be difficultly absorbable form. Then, it remained unsolved what factor is responsible for such a difference between the fish and the rat.

Only qualitative difference in the digestive enzyme system resides in the absence of pepsin and hydrochloric acid in the gold fish which may have some effect on the absorption of calcium in the worm. Further study is needed, however, to make this point clear by using the carnivorous fish provided with pepsin. On the other hand, there is another possibility of showing the low assimilability of calcium in the fish. Since it becomes clear that the calcium of the fish is well equilibrated with the calcium of the surrounding medium, as much calcium will be eliminated as the amount absorbed from the digestive tract, resulting in a considerably low apparent assimilability.

The previous work[1] has shown, on one hand, that the marine fish swallowed some sea water and its calcium appears to be concentrated in the intestine. This study, on the other, presents an evidence to the explanation that high radioactivity in the intestine of fish caught in a sea area which had been contaminated with the fission products, resulted from their contaminated feedstuffs, since in the fission

products were found ^{90}Sr, ^{89}Sr and ^{140}Ba which are in the biochemical behavior similar to ^{45}Ca.

4. Summary

1. A comparative study of the absorption rate of ^{45}Ca from the water with that from a feed, *Rhizodrilus limasus*, has been made by using the gold fish.

2. Irrespectively of different routes for ^{45}Ca up-take, the deposition pattern of ^{45}Ca was found quite similar each other except in the intestine.

3. When ^{45}Ca in a form of absorbed state by the worm was administered to the gold fish, it appeared that only about 8 to 15 per cent was absorbed by the gold fish, a large part being remained in the intestine under the present experimental condition.

4. It has been found that ^{45}Ca absorbed by the worm, was readily utilized to an extent of 79 per cent by the albino rat.

5. The amount of calcium absorbed from the water was overwhelmingly great as compared with that from the feed under the condition studied.

This work was partly supported by the research fund defrayed by the Ministry of Education.

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2) Smith, H. W.: The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol., 83, 480–505 (1930).
3) Tomiyama, T., Ishio, S., and Kobayashi, K.: Absorption of dissolved ^{45}Ca by marine fishes. This publication, p.

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Absorption of Dissolved ^{45}Ca by *Rhizodrilus limasus*

By

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Absorption of Dissolved ^{45}Ca by *Rhizodrilus limasus*

By

Tetuo Tomiyama*, Kunio Kobayashi** and Shinya Ishio***

1. Introduction

Studies have been made previously of the absorption of ^{45}Ca by the fresh and salt water fishes. The results always tend to involve errors due to the difference in individuals. In order to study further on factors governing the absorption of ^{45}Ca by the aquatic animals, search has been made of appropriate organism which is able to absorb with a measurable rapidity and enables us to handle and sample with easiness and homogeneity. A worm, *Rhizodrilus limasus* (Hatai), which is commonly seen in bottom-muds of the domestic sewage was found to be qualified for these requisite. A study has been made on the effect of EDTA-Na_2 on the rate of absorption of ^{45}Ca by this organism.

2. Experimental Method and Results

The G-M Counter of Nuclear model 166 was employed for the measurement of the radioactivity. Sample was weighed and placed on a dish of stainless steel, its bottom area and height being 5 cm^2 and 0.6 cm, respectively, followed by drying under the infrared ray lamp and ashing by the electric muffle furnace. The measurement was made at 1 cm distance from the mica window of the G-M Counter.

i) The absorption of ^{45}Ca ion.

Prior to expose the ^{45}Ca ion, 5 gr of the worm had been kept for four days at 17-20°C. in a well-water, its calcium level being adjusted to 76.8 mg/L by the addition of calcium chloride. Then, the worm was transferred to 200 cc of a radioactive water having the radioactivity of 4,500 cpm/cc. and the calcium level of 76.8 mg/L. At intervals about 0.5 gr sample was taken out, washed for three minutes with 200 cc of the calcium-conditioned well-water, pressed with the filter paper to remove adherent water, and weighed. After the incineration, the radioactivity was measured. Fig. 1 illustrates the rate of increase in the radioactivity. It will be seen that the radioactivity reached to an equilibrium after a 10-hour exposure.

ii) The absorption of ^{45}Ca in the form of calcium ethylendiamine-tetraacetate.

To 200 cc a radioactive well-water containing 15.36 mg calcium was added an equivalent amount of EDTA-Na_2, i.e., 3.84 cc of a 0.1 M EDTA solution, followed by adjustment to pH 7.0. The water thus obtained possessed the radioactivity 4,460

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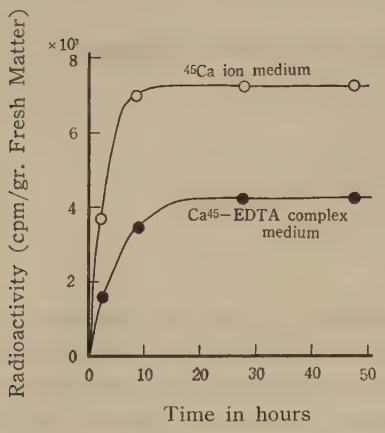

Fig. 1. Absorption of ^{45}Ca ion and ^{45}Ca-EDTA complex in media by *Rhizodrilus limasus* (Hatai).

cpm/cc and almost all the calcium was in combination of EDTA. In this water were placed 5 gr of the worm which had been equilibrated with the same level of calcium as in the radioactive water. At intervals aliquot sample was removed, treated as in the previous experiment, and measured for the radioactivity. The results are shown in Fig. 1. It appears that the calcium salt of EDTA was absorbed as such by the worm. The absorption of ^{45}Ca in this case was nearly half the absorbed amount of ^{45}Ca without EDTA. That really the calcium was less retained at equilibrium was confirmed by the determination of calcium according to the EDTA method. Table 1 shows a good agreement between the analytical value and that calculated from the radioactivity, based on the fact that 56.6 cpm correspond to one microgram of calcium of the radioactive water employed. These data, however, seem not to determine whether the calcium was absorbed as the EDTA complex or as calcium ion from this complex.

Table 1. Amounts of absorbed Ca.

Form of Ca	Radioactivity at equilibrium	Calculated amount of absorbed Ca	Total amounts of Ca
	cpm/gr	γ/g.	γ/g.
Ca ion	7,200	123	125
Ca-EDTA complex	4,200	72	74

iii) The effect of varying the level of EDTA-Na_2 on the absorption of ^{45}Ca.

If EDTA-Ca is assumed to be absorbed as such, no further decrease in the absorption will be resulted from further increasing the concentration of EDTA-Na_2. So, the following experiment has been carried out in a similar way as in the former experiment except the mol ratio of calcium to EDTA being varied between 2 and 1/3. The water used was found to contain the radioactivity of 2,880 cpm/cc and the calcium concentration of 172 mg/L. Fig. 2 illustrates the change in the rate of increase of the radioactivity with variation of EDTA-Na_2 added. In case of no addition of EDTA, the radiocalcium of the worm attained after ten hours to a dynamic equilibrium with that of the water as in the previous experiment. It has been shown, however, that with increasing the concentration of EDTA lowered the absorption of calcium. Irregular curves for the rate of increase of ^{45}Ca was obtained when EDTA-Na_2 was added in amount twice or thrice the equivalent amount to the calcium. The abrupt increase in ^{45}Ca after 5-10 hours may be accounted for by

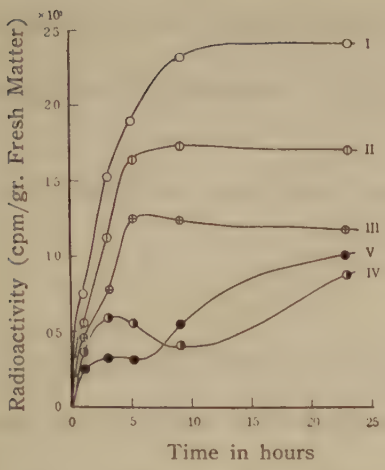

Fig. 2. Influence of Na_2-EDTA upon absorption of ^{45}Ca by *Rhizodrilus limasus* (Hatai).

Water used; Ca, 172 mg/L; Mg, 10.8 mg/L; radioactivity, 2,880 cpm/ml; temperature, 17–20°C. Mol ratio of Ca to EDTA was for curve I, 1:0; curve II, 1:0.5; curve III, 1:1; curve IV, 1:2; curve V, 1:3.

the observation that the death of the worm resulted at about 5 hours exposure. On the other hand, a trial experiment shows that EDTA-Na_2 at 0.27 mMol per L caused the death of the worm after 15 hours while even as high as 10 mM per L of Ca-EDTA gave no effect at all. It will be seen, consequently, that in case where EDTA-Na_2 was added twice the amount equivalent to the calcium EDTA-Na_2 was expected to be present at 3.9 mM per L which is certainly detrimental to the worm.

3. Discussion

In all the experiments it has been observed that the turnover of calcium was taken place every about 10 hours at 17–20°C. It will be of interest to note that this turnover rate was only increased by 4–18% even by increasing the calcium level of the medium by two times as much. It is also noteworthy that the calcium in combination of EDTA appeared to be absorbed by the worm though with a slower rate. This means that EDTA-Na_2 is not highly efficient in the prevention of the contamination with the radioactive isotopes of alkaline earth metals. If it is assumed that calcium salt of EDTA was absorbed as such and that its high molecular weight slowed down the rate of absorption, a further addition of EDTA-Na_2 will not be expected to influence the rate of absorption. It has been shown that this was not the case. However, the experimental result shown in Fig. 2 indicates that a small excess of EDTA-Na_2 apparently detrimental to the worm. So far the present data show it is not able to elucidate the mode of the absorption whether it took place in the form of EDTA-Ca, or in the form of calcium ion which can be assumed to form by decomposing the complex on the surface of the worm if a constant of equilibrium between calcium and the surface be greater than that for the complex.

4. Summary

1. It has been demonstrated that 10 15 hours are required at 17-20°C. for nearly complete replacement of calcium of a worm, *Rhizodrilus limasus*, with that of the medium.

2. The rate of absorption of ^{45}Ca was only slightly influenced by the change in calcium concentration of the medium.

3. The calcium in combination with EDTA appeared to be absorbed by the worm with a smaller rate. It has not been determined of whether EDTA-Ca was

absorbed as such or after dissociation of the complex probably on the surface of the worm.

4. It was observed that a small excess of EDTA-Na_2 manifested a detrimental effect on the calcium absorption by the worm.

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2) Tomiyama, T., Ishio, S., and Kobayashi, K.: Absorption of dissolved ^{45}Ca by marine fishes. This publication, p.

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Absorption of Dissolved ^{45}Ca by Marine Fishes

By

Tetuo Tomiyama , Shinya Ishio and Kunio Kobayashi

Absorption of Dissolved ^{45}Ca by Marine Fishes

By

Tetuo Tomiyama*, Shinya Ishio** and Kunio Kobayashi***

1. Introduction

In the previous paper[1], evidence has been presented that in the fresh water fish the gill is primarily involved in the absorption of ^{45}Ca ion. A very elaborate work of Smith[2] demonstrated that when the marine fish ingested sea water, concentration of magnesium and sulfate ion, took place due to higher rate of the intestinal absorption of water, sodium, potassium and chloride ion. It has already been known that the marine fish drinks sea water in order to meet their water requirement for the metabolism[3]. Accordingly, a study has been made, in comparison with the fresh water fish, of the rate of absorption and deposition pattern of ^{45}Ca in several marine fishes.

2. Experimental method and Results

For the radioactivity measurement was employed the G-M Counter with the "100" scaler of Nuclear Model 166. The sample was placed at a distance of 1 cm from a mica window of the counter. Corrections were applied for the self-absorption and the decay of ^{45}Ca.

i) Deposition of ^{45}Ca in Sphaeroides niphobles and Limanda herzensteini.

A globe fish, *Sphaeroides niphobles*, weighing 30 gr and a flat fish, *Limanda herzensteini*, weighing 129 gr were placed in a vat ($36\times28\times6$ cm^3) containing 4 liters of sea water supplemented with 10 ml of $^{45}Ca\ Cl_2$ (0.020 mc/ml). The sample water gave specific gravity of 1.025 at 14.5°C., radioactivity of 4,930 cpm/ml and calcium content of 476 mg/l. The globe fish, after being exposed for 24 hours, was washed for two minutes with sea water without radiocalcium, sacrificed, and various parts of the body were removed. After weighing these samples, all were dry ashed at 700°C. and their radioactivities were measured as described above. The flat fish was treated in the same way as the globe fish after 1.5-hour exposure to ^{45}Ca ion. The values for the radioactivity as expressed per gram fresh sample was illustrated in Figs. 1 and 2. In both cases, the radioactivity was high in the fin, scale and gill. Remarkable differences in the deposition pattern between two kinds of the fish are noted in the gastro-intestinal tract and the vertebra. The difference in time of exposure to ^{45}Ca ion will account for the difference in deposition of ^{45}Ca in the

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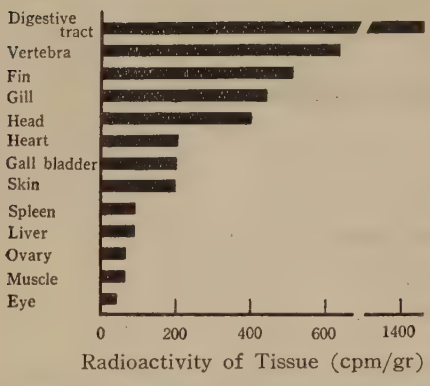

Fig. 1. Absorption of ^{45}Ca dissolved in water by *Sphaeroides niphobles* (Jordan et Snyder).

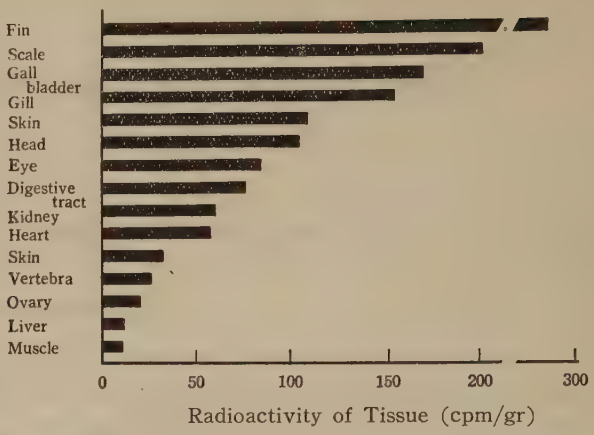

Fig. 2. Absorption of ^{45}Ca dissolved in water by *Limanda herzensteini* (Jordan et Snyder).

vertebra. The highest radioactivity in the digestive tract of the globe fish seems to be due to a habit of swallowing sea water to enlarge its belly in case of emergency. Fairly low radioactivity was found in the liver, ovary and muscle.

ii) Deposition of ^{45}Ca in *Sardinia melanosticta*, *Scomber japonicus*, *Sebastodes inermis*, and *Sphaeroides alboplumbeus*.

Four sardines, *Sardinia melanosticta*, three scombers, *Scomber japonicus*, three scorpaenoid fishes, *Sebastodes inermis*, and five globe fishes, *Sphaeroides alboplumbeus* were placed in an aquarium containing 30 liters of sea water of 12.1‰ chlorinity, added with 30 ml of ^{45}Ca Cl_2 solution and adjusted to pH 8.0. The temperature, calcium content and radioactivity of this sea water was 24°C., 303 mg/l. and 1,916 cpm/ml, respectively. Oxygen in amount of 150 ml per minute was supplied during the experiment. It was observed that the sardine and the scomber showed active movement for few hours followed by gradual weakening, and that the globe fish was very active for many hours while the scorpaenoid fish was sluggish all the time. At intervals, each fish was transferred to a non-labeled sea water, kept for two minutes to remove the surface contamination, and sacrificed. Various organs and tissues were removed, treated as described above, and subjected to the radioactivity measurement.

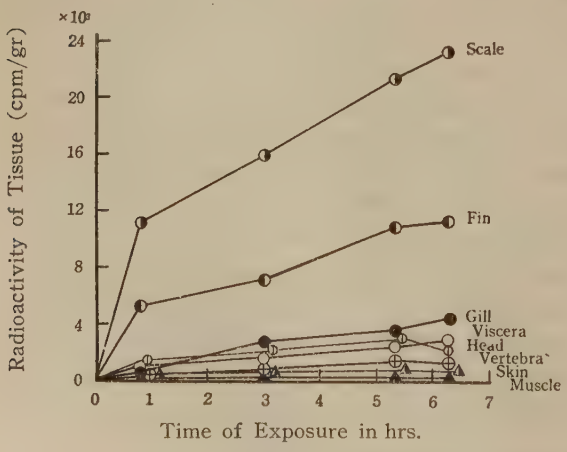

Fig. 3. Rate of absorption of ^{45}Ca from medium by *Sardinia melanosticta* (Temminck et Schlegel).

a. *Sardinia melanosticta*

Fig. 3 shows the rate of increase of the radioactivity per gram fresh matter. It will be seen that the radioactivity was

remarkably high in the scale, fin and gill in this order. It is to be noted that the radiocalcium of the vertebra was rather low and of the muscle was only trace. A comparatively high value recorded in the viscera may be primarily due to presence of the intestine having higher radioactivity.

b. *Scomber japonicus*

The results are shown in Fig. 4. A remarkably high radioactivity was found in the fin. The stomach, gill and vertebra also showed high radioactivity. Low radioactivity was found in the spleen, skin, liver and kidney, and only trace in the muscle.

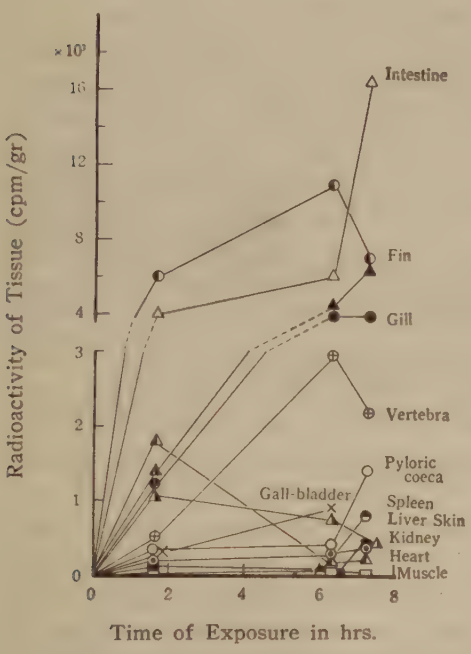

Fig. 4. Rate of absorption of ^{45}Ca from medium by *Scomber japonicus* (Houttuyn).

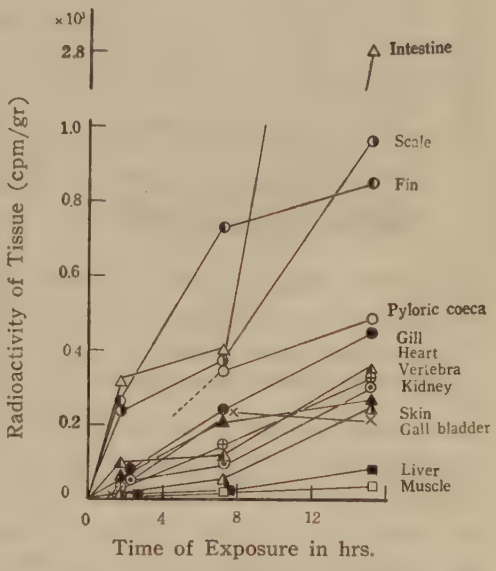

Fig. 5. Rate of absorption of ^{45}Ca from medium by *Sebastodes inermis* (Cuvier et Valencienes).

c. *Sebastodes inermis*

Fig. 5 shows that the radioactivity was very high in the intestine, fin and scale, and was fairly high in the pyloric coeca and gill. Moderate radioactivity was found in the heart, vertebra, kidney, stomach, gall bladder and skin, and the least was in the liver and muscle.

d. *Sphaeroides alboplumbeus*

It will be seen from Fig. 6 that the highest value was in the fin and vertebra, and moderately high value in the gall bladder, gill and gastro-intestinal tract, and low value in the skin, kidney, spleen and heart. The least value was found in the liver and muscle. It is of interest to note that the radioactivity of vertebra was very high in the globe fish devoid of any scale on the skin.

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Excretion of Absorbed ^{45}Ca by Gold Fish

By

Tetuo Tomiyama Kunio Kobayashi and Shinya Ishio

Excretion of Absorbed ^{45}Ca by Gold Fish

By

Tetuo Tomiyama,* Kunio Kobayashi** and Shinya Ishio***

1. Introduction

It has already been reported that ^{45}Ca ion can be absorbed by the fresh water and salt water fish[1,2]. The present study deals with the rate of excretion of the absorbed ^{45}Ca from the various tissues of the radioactive gold fish on keeping it in a non-radioactive water.

2. Experimental Method

Prior to the exposure to ^{45}Ca ion, four gold fishes ranging from 2.4 to 3.6 gr in body weight had been placed in 3 L of a well-water containing 77 mg calcium per L with no supply of feed for five days. This preliminary treatment gives the fishes a complete equilibrium with the same concentration of calcium as that of the radioactive water, i.e., 77 mg/L. Then, the fishes were transferred to another aquarium containing 3 L of a well-water added with 1.5 cc of a $^{45}Ca\ Cl_2$ solution having the radioactivity of 0.19 mc and calcium of 95.2 mg/cc. The above radioactive water was found to possess 4,480 cpm/cc and 77 mg Ca per L. Following a 7-day exposure, the fishes were washed 10 minutes with running water and placed each separately in 1 L of the water containing 77 mg Ca per L. At intervals, each fish was taken up and sacrificed. The various tissues were removed, weighed, dried by the infrared ray lamp and ashed at 700°C. The measurement of radioactivity was carried out as described in a previous paper[1]. Due to no renewal of the non-radioactive water during the experiment, it might be possible that the reabsorption of the excreted ^{45}Ca might be occurred. However, even though it is assumed that the total deposited ^{45}Ca be completely eliminated, this radioactivity amounts to only 50–70 cpm/cc as compared with 4,480 cpm/cc of the radioactive water used for equilibrating the fish with ^{45}Ca. Since the water used throughout the experiment with or without ^{45}Ca possessed the same concentration of calcium, the turnover rate of calcium must be identical no matter whether the water labeled with ^{45}Ca or not. Therefore, it can be seen that the reabsorption of the eliminated ^{45}Ca will not amount to more than 1.0–1.6 per cent of the deposited amount of ^{45}Ca at start of the experiment.

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3. Results and Discussion

Table 1 shows that the decrease in the radioactivity per gr fresh matter with time of exposure to the non-radioactive water. The deposition pattern of ^{45}Ca after being completely equilibrated following a 7-day exposure was nearly the same as reported previously[1], namely, the highest radioactivity was in the scale, and half

Table 1. Decrease in ^{45}Ca expressed as cpm/gr of various fresh tissues during excretion.

Tissue \ Time of culture in days.	0	1	3	6
Scale	56,127	55,706	44,026	30,948
Vertebra	27,466	21,781	16,019	14,247
Gill	27,067	17,979	11,834	10,440
Caudal fin	25,868	23,625	17,844	13,490
Head	12,236	9,851	7,853	6,294
Skin	1,108	1,075	605	338
Muscle	821	658	514	367
Gall bladder	8,351	6,294	2,600	1,426
Digestive tract	4,835	686	1,539	339
Kidney	1,506	667	688	296
Other viscera	355	199	173	125
Total body	14,610	12,105	9,818	8,177
Body weight (g)	2.870	2.394	2.674	3.621

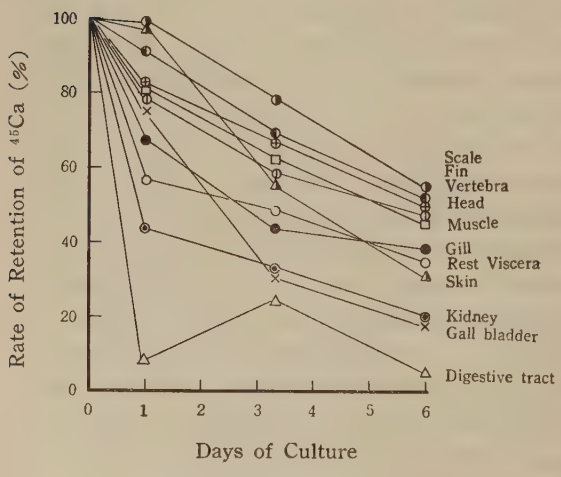

Fig. 1. Rate of retention of ^{45}Ca in various tissues.
◑ Scale, ◐ Fin, ⊕ Vertebra, ① Head, □ Muscle, ● Gill, ○ Rest Viscera, ▲ Skin, ⊙ Kidney, × Gall bladder, △ Digestive tract.

that of the scale was in the vertebra, gill and fin. Fairly high value was observed in the gall bladder and intestine, lower in the kidney and skin, and the lowest in the muscle. The percentage decrease in the radioactivity per gr tissue following transferrence to the non-radioactive water is shown in Fig. 1. It is seen that the percentage decrease was very marked in the intestine, followed by the gall bladder and spleen. Moderate decrease was observed in the skin, viscera, gill and muscle and the least decrease in the vertebra, skull, fin and scale. It is to be noted that gall bladder and kidney showed marked rate

of decrease. This infers that as Smith[3] reported the kidney is responsible for the excretion of calcium. It will be no doubt that the gall bladder also apparently involved in its excretion. Comparatively rapid rate of decrease of the radioactivity in the gill suggests that the gill is not likely a deposition site of calcium but absorption or probably excretory channel for calcium.

Table 2 presents the distribution of ^{45}Ca in the various tissues as expressed in per cent of that of total body. This value was very high in the skull and scale, followed by the fin, gill, vertebra and digestive tract. The lowest was in the muscle, skin and viscera except the digestive tract. It is of interest that some increase of the above value with time was observed in the scale, fin and vertebra, while more or less decrease of the value was seen in the intestine, gill, muscle, skin and viscera. The former series of the tissue showing the increase of the value may be considered to be the deposition tissue in contrast to the latter. It is to be noticed that the gill did not in reality function as a deposition tissue of calcium, although enormous rise of the radiocalcium in the gill appears to be the case.

Table 2. Distribution of ^{45}Ca (%) in various tissues and its change during excretion.

Time of culture in days. / Tissue	0	1	3	6
Scale	27.83	29.28	30.74	26.96
Vertebra	3.34	5.57	4.49	4.95
Gill	7.63	6.35	5.82	5.45
Caudal fin	12.34	16.66	12.04	19.83
Head	34.85	33.11	36.13	32.24
Other bony tissue	9.35	7.16	8.27	9.54
Skin	0.29	0.32	0.26	0.15
Muscle	0.57	0.62	0.54	0.47
Gall bladder	0.19	0.37	0.15	0.10
Digestive tract	3.35	0.42	1.35	0.25
Kidney	0.13	0.04	0.08	0.03
Other viscera	0.14	0.10	0.11	0.08
Total body	100	100	100	100
Total Radioactivity (cpm)	41,972	28,978	26,254	29,607

4. Summary

1. The rate of excretion of ^{45}Ca which was absorbed by the gold fish has been studied.

2. The rate of decrease of ^{45}Ca in the gold fish was low in the scale, fin and vertebra, while very high in the digestive tract, gall bladder and kidney, and moderately high in the muscle, gill, viscera without digestive tract, and skin.

3. The distribution of ^{45}Ca in the various tissues of the gold fish as expressed

in per cent of the total radioactivity was decreased with time in the digestive tract, kidney, gall bladder, gill, viscera without digestive tract, skin and muscle, whereas was increased in the scale, fin and vertebra.

4. It has been shown that the excretion channels for ^{45}Ca are the kidney and gall bladder, and probably the gill.

This study was partly supported by the research grant from the Ministry of Education.

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Reprinted from the Research in the Effects and Influences of the Nuclear Bomb Test Explosions

Excretion of Absorbed ^{45}Ca by *Rhizodrilus limasus*

By

Tetuo Tomiyama, Kunio Kobayashi and Shinya Ishio

Excretion of Absorbed ^{45}Ca by *Rhizodrilus limasus*

By

Tetuo Tomiyama,* Kunio Kobayashi** and Shinya Ishio***

1. Introduction

It has been shown that ^{45}Ca was readily absorbed by a worm, *Rhizodrilus limasus* which is usually found in a muddy deposit of the domestic sewage[1]. The present study deals with the excretion of the absorbed ^{45}Ca after being placed in a non-radioactive water. Furthermore, a study has also been made on the effect of changing the concentration of calcium and ethylendiamine-tetraacetate.

2. Experimental method

The sample worm was placed in a flat porcelain dish and dry ashed in the electric muffle furnace. The measurement of the radioactivity was carried out as described in a previous paper[1]. Fifty gr of the sample worm were kept for 24 hours at 17–20°C. in 3 L of a well-water which possessed the radioactivity of 5,784 cpm/cc and the calcium concentration of 73.6 mg/L. The worm was found to contain 8,980 cpm/gr fresh matter following the exposure. Prior to the excretion experiment, the worm was washed for one minute to remove the surface contamination. Then, 5 gr of the radioactive worm were placed each in five beakers each containing 700 cc of water whose calcium levels were adjusted by treating with sodium oxalate or adding calcium chloride as is shown in Table 1 so as to give a wide range in the ratio of calcium concentration of the radioactive water to that of the non-radioactive water.

Table 1. Concentration of Ca and Mg of waters used.

Group of Expt.	Concentration of Ca (mg/L)	Ratio of Ca of each group to that of group III	Concentration of Mg (mg/L)
I	12.8	$\frac{1}{6}$	13.5
II	26.4	$\frac{1}{3}$	13.5
III	73.6	1	13.5
IV	147.2	2	13.5
V	220.8	3	13.5

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At intervals about 0.5 gr sample was taken out of each beaker, weighed following removal of adhered water by the filter paper, incinerated and subjected to the measurement of the radioactivity.

Similar series of the experiment have been carried out by treating the radioactive worm with waters having different levels of EDTA-Na_2 instead of changing the concentration of calcium. The calcium level of a well-water was adjusted to 84.1 mg/L by the addition of calcium chloride. To each of five lots of 700 cc of this water was added varying volume of M/10 EDTA-Na_2, each lot being adjusted to pH 7.0 with sodium carbonate and filled up to 800 cc. Five grams of the worm which had been exposed to the radioactive water were kept at 17-20°C. in each 800 cc of the EDTA-Na_2-containing water and then the excretion of ^{45}Ca from the worm was followed.

3. Results and Discussion

i) The effect of calcium level on the excretion of ^{45}Ca.

The change in the rate of decrease of ^{45}Ca due to the different levels of calcium is illustrated in Fig. 1. There is a possibility of absorption of the eliminated ^{45}Ca during the experiment. On the other hand, even if the total ^{45}Ca of the worm be totally excreted 24 hours following the transferrence to the non-radioactive water, the eliminated radioactivity would have amounted to 60 cpm/cc which is negligible as compared with 5,784 cpm/cc of the water used for the absorption of ^{45}Ca. Consequently, the reabsorption of the excreted ^{45}Ca will not much influence on the excretion rate. This is also evidenced by the fact that only about 3 per cent of the initial radioactivity was retained in the worm after a 24-hour excretion experiment.

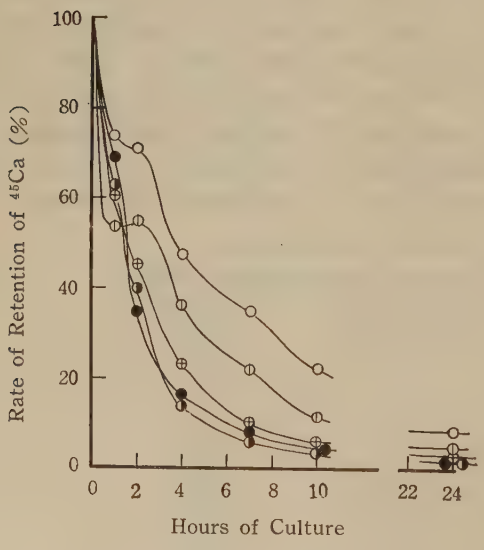

Fig. 1. Change of elimination rate of ^{45}Ca by *Rhizodrilus limasus* with change of calcium concentration in media.

The calcium concentrations for curves I, II, III, IV and V were 12.8, 26.4, 73.6, 147.2 and 220.8 mg/L, respectively.

In case where calcium level was the same as in the absorption of ^{45}Ca, the rate of its elimination was found identical with the absorption rate of ^{45}Ca shown in the previous report[1]. This will be clearly seen from Table 2 which gives the comparable data on the rate of increase and decrease of the radioactivity as expressed in per cent of that at equilibrium. This data also infer that it takes about ten hours to get an over-all turnover of calcium. On changing the calcium concentration of the medium, it was

Table 2. Rate of absorption and elimination of ^{45}Ca by *Rhizodrilus*.

Rate \ Time of culture (hrs)	1	2	4	7	10	24	Concentration of Ca of water (mg/L)
Rate of absorption (%)	31	46	71	90	98	100	76.8
Rate of excretion (%)	41	57	79	84	98	100	73.6

observed that smaller or greater than 73.6 mg/L. the concentration was the slower or quicker the excretion rate resulted. A contradictory and irregular feature, however, was observed within a 2-hr excretion. That is, in lower concentrations the rate of excretion of calcium was comparatively large. This may be accounted for by presuming that ^{45}Ca of the worm was removed due to a sudden decrease in osmotic pressure of the medium prior to the adaptation to lower osmotic pressures.

ii) The effect of EDTA-Na_2 level on the excretion of ^{45}Ca.

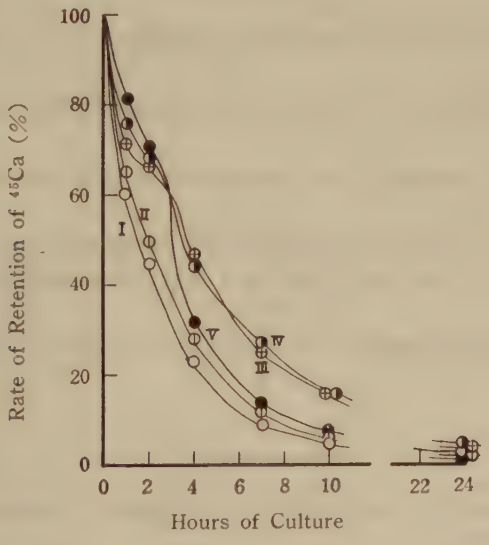

Fig. 2. Change of elimination rate of ^{45}Ca by *Rhizodrilus* with change of Na_2-EDTA concentration in media.

Curve	Mark	[Ca]:[EDTA]	[Ca+Mg]:[EDTA]	Excess[EDTA]mM/L.
I	—○—	1:0	1:0	0
II	—①—	1:0.5	1:0.4	0
III	—⊕—	1:1	1:0.8	0
IV	—◑—	1:2	1:1.6	1.35
V	—●—	1:3	1:2.4	3.19

Further work has been carried out to see whether EDTA-Na_2 be effective in removing ^{45}Ca from the worm. The results obtained are illustrated in Fig. 2. The excretion pattern of ^{45}Ca was expressed as per cent of the initial radioactivity of the worm. Fig. 2 shows that the excretion rate was decreased with increasing the concentration of EDTA-Na_2. Taking into consideration the fact that the excretion rate was decreased with lowering calcium ion of the medium, this decrease is most likely resulted from marked lowering of calcium in the medium. It is to be noted that the addition of EDTA-Na_2 at level of three times equivalent amount resulted in a slower rate at the start whereas an increasing elimination of ^{45}Ca was observed. However, weakening of the worm due to the presence of excess EDTA-Na_2 might give rise to this abnormal result, for it was noticed in this case that death of the worm resulted after 24 hours. These data suggest that EDTA can not be efficiently used for the removal of the contamination of the fish, though it may not be adequate to extend this result of an invertebrate to the decontamination of the teleost. On the other hand, since the rate of elimination of ^{45}Ca seems to be limited by the concentration of calcium ion in the medium, it can generally be concluded that the radiocalcium of the worm can only be removed as a result of replacement.

4. Summary

1. A study has been made of the rate of excretion of ^{45}Ca from a worm, *Rhizodrilus limasus*.

2. The rate of excretion of ^{45}Ca was well comparable to the rate of its absorption.

3. The rate of the excretion was increased with the increase of calcium level in the medium.

4. The addition of disodium ethylendiamine-tetraacetate has been found to make the rate of excretion of ^{45}Ca lower than that without its addition.

This work was partly supported by the research grant from the Ministry of Education.

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1) Tomiyama, T., Kobayashi, K. and Ishio, S.: Absorption of dissolved ^{45}Ca by *Rhizodrilus limasus*. This publication, p.

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Absorption of $^{90}Sr(^{90}Y)$ by Carp

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Tetuo Tomiyama*, Kunio Kobayashi** and Shinya Ishio***

1. Introduction

It becomes well-known that radiostrontiums were invariably present in the fission products produced by the atomic detonation.[1] Fundamental knowledge on the contamination channel for ^{90}Sr has become a problem of pressing import after the Bikini detonation.

Previous data[2] on the deposition pattern of ^{45}Ca by the gold fish were at the time of the Bikini detonation only data to be available in understanding the extent of the contamination of fishes with ^{90}Sr. Consequently, a study similar to that on the absorption of ^{45}Ca has been carried out of the absorption rate of ^{90}Sr by the carp and its deposition pattern in various tissues of the carp. In the present study, several elaborations of the experimental technique have been developed by using ethylurethane to anaesthetize the fish and by obstructing the digestive tract with a mixture of sodium alginate and gips so as to prevent the access of the radioactive water to this channel.

2. Experimental Method

The G-M Counter of Nuclear Model 166 was employed for the measurement of the radioactivity. Aliquot sample was placed on a porcelain dish of 2.8 cm in diameter and 0.7 cm in height. This was dried under the infrared ray lamp, charred on the electric heater and ashed for ten minutes in the electric muffle furnace at about 700°C. The ash was placed at 1.2 cm distance from the mica window of the G-M Counter. Correction was applied for the self-absorption depending on weight of the ash.

In order to apply the isotope with safety to the absorption experiment, it was tried to put the carp under anaesthetic. Gaarder[3] reported that a 0.4% solution of ethylurethane was appropriate for a study of the energy metabolism of the fish. It has been found, however, that a 0.25% solution of ethylurethane was effective enough to stop the movement. Though the respiratory movement was observed to be reduced to about one-half of the normal, the fish was observed to keep this reduced rate constant many hours following the administration of ethylurethane. Accordingly, prior to start the absorption experiments the carp was always subject to anaesthetization with appropriate volume of a 0.25% ethylurethane solution for 2 hours.

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To enable us to obtain enough blood sample to determine the radioactivity the carp was used whose body weight ranged from 600-800 gr. In order to analyze for probable absorption channels for $^{90}Sr(^{90}Y)$, the following procedures have been developed. Firstly, the determination was made of the absorption pattern of the carp which was subjected to no treatment except anaesthetization. In this case, the absorption will take place at any part of the fish body. Secondly, the absorption experiment was made on the carp whose oesophagus was obstructed by impregnating with "algix", a commercial preparation of a mixture of sodium alginate and gips. Due to this treatment the absorption will have to occur at any part except through the digestive tract. Thirdly, the estimation was made of the absorption from either the head part comprising the gill or from the remainder part by using an apparatus shown in Fig. 1. When only the head part was exposed to $^{90}Sr(^{90}Y)$, the absorption will take place mainly at the gill and the digestive tract. In case where the remainder part was exposed, only the surface will be attributable to the channel. In these ways it can be realized to get an information of what channel is of importance among various possible cases. Followings are protocols of the various absorption experiment.

i) The absorption by the intact fish.

Six anaesthetized carps were placed in an aquarium made of polyvinyl sheet containing 15 L of a well-water supplemented with 37.5 g ethylurethane and 30 cc of a $^{90}Sr(^{90}Y)Cl_2$ solution, the strontium concentration and the radioactivity being 0.3 mg/L and 1.2×10^6 cpm/cc., respectively. This radioactive well-water contained the radioactivity of 2,400 cpm per cc and the calcium- and the magnesium-concentration of 32 mg and 9 mg per L, respectively. The temperature ranged from 13.5 to 15.5°C., and enough aeration was applied throughout the experiment. At intervals each fish was taken out, washed thoroughly in a streaming well-water for one minute. About five cc of the blood were drawn out by a syringe directly from the heart. Then, various tissues were removed and measured for the radioactivity according to the procedure afore-mentioned.

ii) The absorption by the fish with obstructed oesophagus.

The fresh water fish has been believed not to swallow the water. In the present anaesthetized condition, however, there may happen to occur a penetration of the water into the oesophagus. The prevention of this leakage requires ordinarily the ligature at the oesophagus. It is difficult, however, to carry out the ligature without injury which may complicate the absorption pattern. Accordingly, an attempt was made to stop the oesophagus by the impregnation, and it was finally found that the following procedure was always successful. Into the oesophagus a polyvinyl tube of 7 mm in the inner diameter was inserted. Into this tube was inserted another polyvinyl tube of 5 mm in outer diameter containing about 1 g of the "algix", i. e., a mixture of sodium alginate and gips. Then, the "algix" was forced into the oesophagus by pushing a gummy ball which was attached to the end of the smaller polyvinyl tube. The carp thus treated was kept for 2 hours at 14°C. in the same radioactive water as was used in the former experiment, the aeration of the water being constantly made.

iii) The absorption from the head part.

The narcotized fish was kept in a position as shown in Fig. 1 by two gummy membranes which were fixed with the respective bell-jars. In this way, any leakage of the isotope from any one of the compartment can be readily removed by running water. So that any contamination of the other compartment will never occur. Of 3 L of the well-water containing 7.5 gr ethylurethane, 2 L were introduced into the compartment B in Fig. 1 and one liter added with a $^{90}Sr(^{90}Y)Cl_2$ solution was introduced into the compartment A where bubbling of air was constantly supplied. The radioactivity of the water in A was 2,100 cpm/cc. The aquarium was kept at 13°C. by supplying a running water. Twenty-five hours following the exposure, the fish was removed from the apparatus and washed with the running water for one minute. The radioactivity of the various tissues was measured as usual.

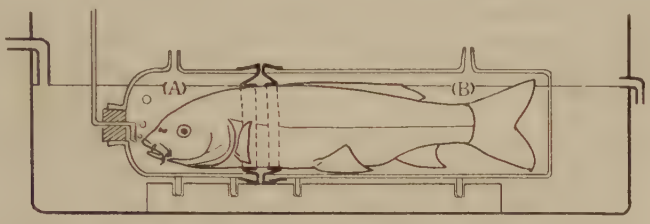

Fig. 1. Apparatus for determination of absorption channel.

iv) The absorption from the surface.

The experiment was carried out just as the former one except that the compartment B was added with a water containing the radioactivity of 3,650 cpm/cc. and the compartment A was filled with the non-radioactive water.

3. Results and Discussion

i) The absorption by the intact fish.

The radioactivity per gr of the various tissues of each carp exposed at intervals was presented in Table 1. It will be seen that even after 30 minutes the radioactivity was already found in the blood and the various tissues except the muscle and gall bladder. Table 1 shows that the various tissues can be divided into two groups with respect to the rate of increase of the radioactivity. That is, the increase was observed at least over a 25-hr period in the scale, vertebra, air bladder and gall bladder, while in the other tissues an abrupt increase took place within several hours after the exposure, followed by almost no increase. In order to see the deposition pattern, the ratio of the radioactivity of the various tissues to that of the blood was calculated for each time interval. The calculated ratios are shown in Table 2. Values given in Table 2 indicate that the gill, caudal fin, scale and vertebra are tissues which are capable of enriching by some way $^{90}Sr(^{90}Y)$ from the blood. It will be noticed that the caudal fin and digestive tract showed a great value within 2 hr-exposure. A high value for the caudal fin infers that this tissue is very active

Table 1. Absorption of $^{90}Sr(^{90}Y)$ by intact carp.
Values given are expressed as cpm/gr of fresh tissues. Water used: radioactivity, 2,400 cpm/cc; urethane, 0.25%; temperature, 13.5–15.5°C.

Tissue \ Time of exposure in hrs.	0.5	1.5	2.0	5.5	14.5	25.0
Blood	5	25	31	87	45	58
Gill	78	205	357	7,791	1,220	2,175
Caudal fin	266	727	846	1,234	814	1,810
Scale	32	59	116	379	294	697
Vertebra	10	54	89	199	158	301
Air bladder	8	17	21	65	56	84
Heart	7	19	33	72	47	54
Skin	9	35	37	50	36	35
Kidney	7	12	15	49	34	50
Liver	1	26	29	66	18	44
Spleen	4	15	11	52	24	31
Gall bladder	0	5	8	18	12	32
Gonad	♂1	♀6	♂11	♀28	♀19	♂17
Muscle	0	7	10	17	10	15
Gastro-intestinal tract Anterior	285	311	212	102	60	35
Gastro-intestinal tract Posterior	196	171	168	81	158	51
Body weight (g)	752	791	634	785	580	624

Table 2. Relative values of radioactivity as compared with that of blood.

Tissue \ Time of exposure in hrs.	0.5	1,5	2.0	5.5	14.5	25.0
Blood	1.0	1.0	1.0	1.0	1.0	1.0
Gill	15.6	8.2	11.5	89.6	27.1	37.5
Caudal fin	53.2	29.1	27.3	14.2	18.2	31.2
Scale	6.5	2.4	3.7	4.4	6.5	12.0
Vertebra	2.0	2.2	2.9	2.3	3.5	5.2
Air bladder	1.6	0.7	0.7	0.8	1.2	1.5
Heart	1.4	0.8	1.1	0.8	1.0	0.9
Skin	1.8	1.4	1.2	0.6	0.8	0.6
Kidney	1.4	0.5	0.5	0.6	0.8	0.9
Liver	0.2	1.0	0.9	0.8	0.4	0.8
Spleen	0.8	0.6	0.4	0.6	0.5	0.5
Gall bladder	0	0.2	0.3	0.2	0.3	0.6
Gonad	♂0.2	♀0.2	♂0.4	♀0.3	♀0.4	♂0.3
Muscle	0	0.3	0.3	0.2	0.2	0.3
Gastro-intestinal tract Anterior	57.0	12.4	6.8	1.2	1.3	0.6
Gastro-intestinal tract Posterior	39.2	6.8	5.4	0.9	3.5	0.9

in the turnover of calcium, but a similar high value for the digestive tract may be due to the ingested radioactive water at start of the experiment.

ii) The absorption by the fish with obstructed oesophagus.

That the "algix" was able to completely obstruct the oesophagus was evidenced by the observation that about two-thirds of the column near to the pharynx absorbed water while the other part of the column remained as powder and that the radioactivity in the "algix" column was found lower than that in the intestine. Table 3 shows the radioactivity in the various tissues of the carp with the obstructed oesophagus as compared with that of the intact carp. From Table 3 it will be seen that the value in the caudal fin, gill, scale and vertebra was rather higher while that in the viscera particularly the digestive tract was far lower. A comparison between the absorptions by the intact carp and by the carp with the closed oesophagus indicates that the absorption through the digestive tract is of no importance.

iii) The absorption from the head part and from the surface.

The results obtained are given in Table 4. The absorbed radioactivity from the head part was nearly the same as that absorbed by the intact carp and was

Table 3. Absorption of $^{90}Sr(^{90}Y)$ by carp with obstructed oesophagus. Values given are expressed as cpm/gr of fresh tissues. Water used; radioactivity, 2,400 cpm/cc; urethane, 0.25%; temperature, 14°C.

Treatment of oesophagus		No obstruction	With obstruction
Time of exposure (hr) / Tissue		2.0	2.0
Blood		31	29
Gill		357	510
Caudal fin		846	834
Scale		116	306
Vertebra		89	118
Air bladder		21	8
Heart		33	42
Skin		37	57
Kidney		15	12
Liver		29	15
Spleen		11	7
Gall bladder		8	7
Gonad		♂11	♂4
Muscle		7	13
Gastro-intestinal tract	Anterior	212	37
	Posterior	168	66
"Algix"	Outside	—	44
	Central	—	30
	Inside	—	48
Body weight (g)		634	597

Table 4. Absorption of $^{90}Sr(^{90}Y)$ from head part and remainder part. Radioactivities (cpm/cc) of waters used in three experiments were 2,400 for intact carp, 2,100 for exposure of head and 3,650 for exposure of remainder part. All the values given are expressed as cpm/gr of fresh tissues and corrected for difference in radioactivity of water used, viz., based on radioactivity at a level of 2,400 cpm/cc.

Site of absorption	Total body	Head part	Remainder part
Time of exposure (hr) / Tissue	25	25	25
Blood	58	63	5
Gill	2,175	1,845	41
Caudal fin	1,810	781	136
Scale	697	902	38
Vertebra	301	229	7
Air bladder	84	129	3
Heart	54	66	2
Skin	35	56	6
Kidney	50	47	0
Liver	44	58	2
Spleen	31	30	0
Gall bladder	32	38	3
Gonad	♂17	♂7	♀3
Mnscle	15	11	3
Gastro-intestinal tract (Anterior)	35	88	3
Gastro-intestinal tract (Posterior)	51	172	1
Body weight (g)	624	585	603

very high as compared with that from the surface of the body. From data presented in Table 2 it appears that the deposition of the radioactivity in the caudal fin may occur through the skin and not from the blood since the rate of increase in the radioactivity was as equally high in the skin as in the blood. Data given in Table 4 show, however, that this is not the case, since when radioactivity was absorbed from the skin, it was deposited on the caudal fin to a far smaller degree than when absorbed from the gill.

As the most probable absorption channel which is located at the head part, the gill, mouth cavity and pharynx shall be considered. Among them the gill is attributable to the most important channel, since a rapid increase and high level of the radioactivity on exposure to $^{90}Sr(^{90}Y)$ was always observed in the gill. The present conclusion is in harmony with that obtained in the previous report on the absorption channel for calcium. It will be quite reasonable that these elements which are similar each other in chemical behavior and the deposition pattern in the body, is absorbed through the identical route.

4. Summary

1. A study has been made of the absorption and deposition of $^{90}Sr(^{90}Y)$ ion by the carp.

2. It has been demonstrated that $^{90}Sr(^{90}Y)$ ion was absorbed primarily through the gill, to a small extent through the skin and not absorbed in a measurable amount through the digestive tract.

3. The rate of absorption of $^{90}Sr(^{90}Y)$ ion by the carp was fairly high, i.e., after 30-minute exposure some radioactivity was already found in most tissues except the muscle and gall bladder.

4. The deposition pattern similar to that of ^{45}Ca was seen in the carp following the absorption of $^{90}Sr(^{90}Y)$ from the water, namely, the radioactivity was high in the gill, caudal fin, scale and vertebra in this order, followed by the blood, air bladder, heart, skin, liver, kidney and spleen, and was the least in the muscle.

This work was partly supported by the research grant from the Ministry of Education.

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Distribution and Excretion of Intramuscularly Administered $^{90}Sr(^{90}Y)$ in Carp

By

Tetuo Tomiyama Kunio Kobayashi and Shinya Ishio

Distribution and Excretion of Intramuscularly Administered $^{90}Sr(^{90}Y)$ in Carp

By

Tetuo Tomiyama*, Kunio Kobayashi** and Shinya Ishio***

1. Introduction

In the preceding paper[1] it has been shown that the carp was capable of absorbing $^{90}Sr(^{90}Y)$ ion primarily through the gill, and that the deposition pattern of $^{90}Sr(^{90}Y)$ was quite similar to that of ^{45}Ca. The present study has been undertaken to find the excretion channel for the absorbed $^{90}Sr(^{90}Y)$. Prior to start of the experiment it is naturally of necessity to afford the fish with an abundant deposition of $^{90}Sr(^{90}Y)$. Attempt was made to administer intramuscularly a large dose of $^{90}Sr(^{90}Y)$ and it was found that the deposition pattern was essentially similar to that which was deposited after the absorption from the water. Hence, an excretion experiment has been carried out on the carp subjected to the intramuscular administration of $^{90}Sr(^{90}Y)$.

2. Experimental Method and Results

A carp weighing 540 gr was anaesthetized by keeping it in a 0.25% ethylurethane solution for 2 hours. Then, it was fixed at just hinder part of the operculum by two thin gummy membranes in the apparatus illustrated in the preceding report[1]. Into both chambers were introduced a well-water containing 0.25% ethylurethane, and oxygen was supplied at a rate of 100–150 cc per minute. Two cc of a neutralized solution of $^{90}Sr(^{90}Y)Cl_2$ containing the radioactivity of 1.8×10^6 cpm and strontium of 0.45 microgram were injected intramuscularly at a dorsal point just between two gummy membranes. This resulted in 3,300 cpm/gr body weight. Running water was introduced into the aquarium to maintain the temperature at 13°C. and also to remove any trace of $^{90}Sr(^{90}Y)$ leaked out of the injected puncture. Twenty hours following the injection the blood was sampled directly from the heart and the various tissues were removed for the measurement of the radioactivity whose procedure was the same as previously described[1]. The deposition pattern thus obtained and the value for the ratio of each specific activity to that of the blood are presented in Table 1. It is seen that the deposition pattern in this case was qualitatively similar to that derived through the absorption from the water.

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It will be noticed again that a relatively high radioactivity was found in the spleen and kidney. This will be discussed later on.

Table 1. Distribution of $^{90}Sr(^{90}Y)$ following intramuscular administration. $^{90}Sr(^{90}Y)$ administered, about 1.8×10^6 cpm per 540 gr; time of exposure, 20 hrs.; temperature, 13–13.5°C; urethane, 0.25%.

Tissue	Radioactivity (cpm/gr)	Ratio to blood	Distribution (%)
Blood	718	1.00	1.52
Gill	15,420	21.48	10.18
Caudal fin	8,657	12.06	3.66
Scale	11,490	16.00	14.56
Vertebra	5,935	8.27	6.27
Air bladder	1,233	1.72	0.36
Spleen	1,202	1.67	0.06
Kidney	1,166	1.62	0.18
Heart	599	0.83	0.03
Gall bladder	527	0.73	0.08
Liver	506	0.70	0.33
Skin	481	0.67	0.71
Muscle	180	0.25	2.85
Testis	97	0.14	0.31
Gastro-intestinal tract Anterior	379	0.53	0.08
Gastro-intestinal tract Posterior	368	0.51	0.08

The excretion experiment was carried out according to the same procedure as above-described. Into a carp weighing 642 gr was injected 2 cc of a $^{90}Sr(^{90}Y)Cl_2$ solution ten times as much concentrated in the radioactivity as was used in the foregoing experiment, resulting in 31,150 cpm/gr body weight. Twenty-four hours following the injection the radioactivity was measured of the various tissues and of waters contaminated with renal or extrarenal excretion. The excreted radioactivity was measured as follows. Aliquots, about one-fiftieth of the total volume of the waters, were added with 0.2 gr strontium nitrate and precipitated with ammonium oxalate. The precipitates were separated with filter paper, dried and placed in the porcelain dish together with the filter paper. After incineration at 700°C., the measurement of its radioactivity was made as described in a previous paper[1]. It was confirmed by a preliminary experiment that the above coprecipitation procedure gave a complete recovery of ^{90}Sr and 90% recovery of ^{90}Y.

It is to be noted that the measurement of radioactivity was carried out 48 hours following the injection of $^{90}Sr(^{90}Y)$. Hence, it follows that if ^{90}Sr showed a different deposition pattern from ^{90}Y, the measured radioactivity would not indicate the real deposition pattern of these isotopes because the half-life of ^{90}Y is only 60 hours in contrast to that of 25 years for ^{90}Sr. Therefore, determinations were

made of the ratio of ^{90}Sr to ^{90}Y in the various tissues by using an aluminium absorber of 129 mg per cm^2. Table 2 shows the radioactivity per gr fresh matter for the various tissues 24 hours following the intramuscular injection of $^{90}Sr(^{90}Y)$. It also includes the ratio of the radioactivity of the various tissues to that of the blood. The deposition pattern was found in this experiment similar to the former experiment. The values for the ratio of tissues to the blood showed in the latter experiment comparatively higher values for the scale, spleen and kidney and also

Table 2. Distribution of $^{90}Sr(^{90}Y)$ following intramuscular administration of its large dose.
$^{90}Sr(^{90}Y)$ administered, about 2×10^7 cpm per 642 gr; time of exposure, 24 hrs.; temperature, 13–13.5°C; urethane, 0.25%.

Tissue			Radioactivity (cpm/gr)	Ratio to blood	^{90}Sr (%)	^{90}Y (%)
Blood			2,882	1.00	62	38
Gill			50,565	17.55	55	45
Caudal fin			39,989	13.88	57	43
Scale			73,627	25.55	64	36
Vertebra			28,905	10.03	56	44
Air bladder			4,970	1.72	61	39
Spleen			10,252	3.56	1	99
Kidney			21,118	7.33	2	98
Heart			2,969	1.03	42	58
Gall bladder			2,790	0.97	68	32
Liver			3,759	1.30	18	82
Skin			3,737	1.30	31	69
Muscle			637	0.22	44	56
Ovary			3,582	1.24	38	62
Gastro-intestinal tract	Anterior	Tissue	1,751	0.61	57	43
		Content	1,518	0.53	56	44
	Posterior	Tissue	1,756	0.61	59	41
		Content	1,855	0.64	64	36

high for ovary as compared with testis in the former experiment. It is of noteworthy that the radioactivity of the spleen and kidney were exclusively due to ^{90}Y, and that those due to ^{90}Y of the liver, skin, ovary, heart aud muscle amounted to over 50% of the total. On the contrary, ash-rich tissues such as the scale, caudal fin, vertebra and gill were comparatively rich in ^{90}Sr. The radioactivity which was excreted renally as well as extrarenally, and the ratio of ^{90}Sr to ^{90}Y were given in Table 3. It will be seen that about 1% of the administered $^{90}Sr(^{90}Y)$ was eliminated during 24 hours and that about 84% of the total excreted radioactivity was eliminated through the renal route and the remainder was likely through the gill.

Table 3. Excretion of ^{90}Sr and ^{90}Y.

Excretion channel	Extrarenal	Renal
Total excreted radioactivity (cpm)	29,700	151,450
Per cent of total radioactivity	16	84
^{90}Sr (%)	70	71
^{90}Y (%)	30	29

The distribution of the radioactivity between the corpuscles and the plasma was also determined. 5 cc of the blood was mixed in the syringe with 0.5 cc of a 5% sodium citrate. The separation of the corpuscles was made by a 10-minute centrifugation at 3,000 rpm. Both the corpuscles and the plasma are subjected to the measurement of the radioactivity. The values per gr of fresh matter are presented in Table 4. The data show that the specific radioactivity was always about six times as much in the plasma as in the corpuscles, and the ratio of ^{90}Sr to ^{90}Y was lower in the plasma than in the corpuscles.

Table 4. ^{90}Sr and ^{90}Y in plasma and red blood cells following intramuscular administration.

Mode of administration	Plasma	Cells	Distribution		Ratio, ^{90}Sr : ^{90}Y	
			Plasma	cells	Plasma	cells
20 hrs following injection of dose, 3,300 cpm/gr	cpm/gr 1,029	cpm/gr 189	cpm/gr of blood 648	70	—	—
24 hrs following injection of dose, 31,150 cpm/gr	3,028	414	1,908	153	58 : 42	68 : 32
25 hrs Exposure to water of 2,400 cpm/cc	107	16	67	5.8	—	—
Volume of plasma and cells	63%	37%				

3. Discussion

The behaviours of ^{90}Sr and ^{90}Y in the deposition in the various tissues are of interest. It is quite reasonable that ^{90}Sr was deposited in the identical way as calcium. The data that ^{90}Sr was rich in the gall bladder infers that this organ may partly be involved in the excretion of ^{90}Sr. Similarly, the high and exclusive distribution of ^{90}Y in the kidney and spleen suggests that these organs play an important rôle in the metabolism of ^{90}Y.

The analysis of the renal or extrarenal excretion for ^{90}Sr or ^{90}Y showed 70%

of the total radioactivity to be due to ^{90}Sr, suggesting that ^{90}Y might not be so readily excreted as ^{90}Sr. Smith[2] reported of the evidence that sodium, potassium and chloride ion was excreted through the gill. It has been demonstrated in the present study that both ^{90}Sr and ^{90}Y appeared to be partly excreted by way of the gill. The ^{90}Y excreted was found to amount to 30% of the total radioactivity. It is of course doubtful whether the ^{90}Y was excreted at this level since some of the measured ^{90}Y was originated from ^{90}Sr during the course of the experiment.

4. Summary

1. The deposition pattern, in the various tissues of the carp, of $^{90}Sr(^{90}Y)$ which was administered intramuscularly has been found to be similar to that which was absorbed from the surrounding water.

2. The ^{90}Y and ^{90}Sr were estimated from the radioactivity of the various tissues by using the appropriate absorber.

3. It has been found that ^{90}Sr was higher than ^{90}Y in ash-rich tissues such as the caudal fin, scale, vertebra, and gill, and also in the blood, air bladder, gall bladder and digestive tract. The ^{90}Y has been found higher than the ^{90}Sr in most internal organs, skin and muscle. It is of interest that the kidney and spleen were very rich in ^{90}Y and nearly free from ^{90}Sr.

4. The intramuscularly administered $^{90}Sr(^{90}Y)$ has been found to be excreted as much as 84% of the total excreted radioactivity mainly through the renal route, the remainder being excreted through the gill.

5. It has been shown that ^{90}Y appeared to be excreted to a less degree than ^{90}Sr, and that ^{90}Sr seemed to be partly excreted through the gall bladder.

6. The radioactivity of the blood was largely due to ^{90}Sr in the plasma.

This work was partly supported by the research grant from the Ministry of Education.

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Absorption of $^{32}P\ O_4$ Ion by Carp

By

Tetuo Tomiyama Kunio Kobayashi and Shinya Ishio***

Absorption of $^{32}P\,O_4$ Ion by Carp

By

Tetuo Tomiyama*, Kunio Kobayashi** and Shinya Ishio***

1. Introduction

It has previously been shown that ^{45}Ca ion as well as ^{90}Sr (^{90}Y) ion was absorbed by the fresh water fish from the water mainly through the gill[1,2]. It will be of interest, accordingly, to investigate of whether $^{32}P\,O_4$ ion be absorbed by the carp from the surrounding water, and to determine, if it is really absorbed, the site which is involved in its absorption and the deposition in various tissues.

2. Experimental Method

Experimental procedure employed was nearly identical with that in the previous report[2]. Samples were all dried by the infrared ray lamp, charred on the electric heater and reduced to ashes at about 700°C for ten minutes. No loss of the radioactivity was observed during this procedure. The measurement of radioactivity was carried out by employing the G-M Counter and "100" scaler of Nuclear Model 166. Corrections were applied for the self-absorption as well as the decay during the experimental period.

i) Absoption of $^{32}P\,O_4$ ion by intact carp.

To 15 L of a well-water was added 37.5 gr ethylurethane and about 1 cc of a carrier free $H_3\,^{32}P\,O_4$ solution having about 6×10^7 cpm/cc, followed by adjustment to pH 7.0. Four carps weighing 619-635 gr in body weight which were narcotized in a 0.25% urethane solution, were kept at 12-13.5°C in the above cited radioactive water containning 3,660 cpm/cc. Oxygen was supplied at a rate of 100-150 cc per minute. At intervals each carp was washed for one minute. After about 5 cc of the blood being drawn from the heart, the various tissues were removed and measured for the radioactivity.

ii) Absorption of $^{32}P\,O_4$ ion by carp with obstructed oesophagus.

One gr of "algix", a mixture of sodium alginate and gips, was administered to an anaesthetized carp weighing 663 gr by the procedure described in the previous report[1], and immersed for 2 hours at 13°C. in the same radioactive water as was used previously with a constant supply of oxygen. The aftertreatment of the fish was identical with that of the former experiment. It was observed on dissection that the oesophagus was completely impregnated with the "algix".

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iii) Absorption of $^{32}PO_4$ ion from the head part.

Use was made of the apparatus which was illustrated in the previous paper[2]. Eight hundred cc of the radioactive water containing 2,460 cpm/cc were introduced into a chamber which accommodated the head part of an anaesthetized carp. The apparatus was placed in an aquarium and was supplied with streaming water to make a temperature at 13°C. and also to remove any radioactive water which might happen to leak out. After the carp being kept at the exposure for 25 hours, it was washed for one minute and treated as in the experiment i).

iv) Absorption of $^{32}PO_4$ ion from the surface except the gill.

The experiment was carried out just in the identical way as above-described except that the remainder part instead of the head part was exposed to $^{32}PO_4$ ion whose radioactivity being 3,450 cpm/cc.

3. Results and Discussion

i) Absorption of $^{32}PO_4$ ion by intact carp.

Data presented in Table 1 show that $^{32}PO_4$ ion in the surrounding water can readily be absorbed by the carp. In contrast to the behaviour of ^{45}Ca and ^{90}Sr (^{90}Y) in the deposition pattern, $^{32}PO_4$ was distributed in the blood, heart, liver, kidney and digestive tract not less than in the scale. Data of high radioactivity in the

Table 1. Absorption of $^{32}PO_4$ by intact carp.
Water used; radioactivity, 3,660 cpm/cc; urethane, 0.25 %; temperature, 12-13.5°C.; Values given are expressed as cpm/gr of fresh tissues.

Tissue \ Time of exposure in hrs.			2	8	16	25
Blood			40	72	107	389
Gill			121	225	305	790
Scale			3.3	12.7	30.9	117
Caudal fin			85	105	117	189
Vertebra			1.0	6.3	14.4	25.8
Kidney			4.5	27.2	57.3	150
Liver			6.6	47.5	138	341
Heart			85	216	493	178
Spleen			0	5.2	9.8	78
Air bladder			8.0	13.1	18.3	56.0
Gall bladder			0	5.9	13.8	20.8
Gonad			♂ 0	♀ 2.1	♂ 6.7	♂ 15.5
Skin			20.6	26.7	35.2	52.3
Muscle			0	1.0	2.9	8.9
Gastrointestinal tract	Anterior	Tissue	3.1	102	205	161
		Content	104	1,724	3,205	327
	Posterior	Tissue	10.2	32.5	65.0	94.0
		Content	5.7	1,524	3,780	240
Body weight (g)			619	647	658	635

heart and liver infers that ^{32}P might be incorporated in some important compound for the metabolism such as ATP. A remarkable increase of ^{32}P in the blood is particularly noticed. No further study on the distribution of ^{32}P between the plasma and the corpuscles has been made in this experiment. However, as will be reported later, when $^{32}P\,O_4$ ion was administered intramuscularly, it was found that the blood corpuscle possessed ^{32}P about twice as much as the plasma. This will suggest that $^{32}P\,O_4$ which was absorbed through the gill may be incorporated to a considerable degree in the corpuscles.

In order to see the extent of accumulation of the radioactivity in these tissues, a relative value is obtained for each tissue by dividing its radioactivity by that of the blood. The values given in Table 2 show that only such tissues as the gill,

Table 2. Relative values of radioactivity as compared with that of blood.

Tissue \ Time of exposure in hrs.			2	8	16	25
Blood			1.00	1.00	1.00	1.00
Gill			3.03	3.13	2.85	2.03
Scale			0.08	0.18	0.29	0.30
Caudal fin			2.13	1.46	1.09	0.49
Vertebra			0.03	0.09	0.13	0.07
Kidney			0.11	0.38	0.54	0.39
Liver			0.17	0.66	1.29	0.88
Heart			2.11	2.99	4.61	0.46
Spleen			0	0.07	0.09	0.20
Air bladder			0.20	0.18	0.17	0.14
Gall bladder			0	0.08	0.13	0.05
Gonad			♂ 0	♀ 0.03	♂ 0.06	♂ 0.04
Skin			0.52	0.37	0.33	0.13
Muscle			0	0.01	0.03	0.02
Gastrointestinal tract	Anterior	Tissue	0.08	1.42	1.92	0.41
		Content	2.60	23.94	29.95	0.84
	Posterior	Tissue	0.26	0.45	0.60	0.24
		Content	0.14	21.17	35.33	0.62

caudal fin and heart were higher than unity. It becomes apparent that $^{32}P\,O_4$ ion is absorbed through the gill, transported by the blood, and may be used for the formation of the energy-rich bond in the heart. Very low radioactivity in the muscle was unexpected. It can be presumed, however, that under anaesthetized condition the incorporation of $^{32}P\,O_4$ into ATP or the alike may be negligibly small due to no muscular work being performed. As compared with a regular increase of ^{32}P in the blood as well as the whole body, was observed an irregularity in the radioactivity of the heart which may be attributable to a certain subtle change in the heart activity under the present condition. It is of noteworthy that ^{32}P was deposited with a rapid rate in the caudal fin while deposited slowly in the

scale and vertebra. A comparatively high radioactivity in the kidney means that this organ is undoubtedly involved in the excretion. An abrupt rise in the radioactivity in the spleen after a 25-hour exposure may be accounted for by the destruction of the corpuscles which absorbed fairly large amount of ^{32}P.

ii) Absorption of $^{32}PO_4$ ion by carp with obstructed oesophagus.

The fact that the radioactivities of the "algix" column formed in the oesophagus were 17, 0 and 24 in the exterior, middle and interior part, respectively, indicates that $^{32}PO_4$ ion could not be ingested to any extent by the complete obstruction. Table 3 shows a comparison of the present data with that of the absorption

Table 3. Absorption of $^{32}PO_4$ by carp with obstructed oesophagus.
Values given are expressed as cpm/gr of fresh tissue.
Water used; radioactivity, 3,660 cpm/cc; urethane 0.25%; temperature, 13°C.

Treatment of oesophagus			No obstruction	With obstruction
Tissue \ Time of exposure (hr)			2	2
Blood			40	51
Gill			121	136
Scale			3.3	7.8
Caudal fin			85	92
Vertebra			1.0	2.1
Kidney			4.5	3.6
Liver			6.6	7.7
Heart			85	512
Spleen			0	2.3
Air bladder			8.0	3.3
Gall bladder			0	1.7
Gonad			♂ 0	♂ 1.9
Skin			20.6	19.4
Muscle			0	1.2
Gastrointestinal tract	Anterior	Tissue	3.1	2.6
		Content	104	—
	Posterior	Tissue	10.2	4.5
		Content	5.7	8.9
"Algix"	Outside		—	17.2
	Central		—	0
	Inside		—	23.9
Body weight (g)			619	663

of $^{32}PO_4$ ion by the intact carp two hours following the exposure. It is seen that the prevention of entering the radioactive water into the digestive tract did not lower the deposition of ^{32}P in the various tissues. These data lead us to the conclusion that in the carp, probably the fresh water fish in general, the digestive tract does not play an active part in the absorption of $^{32}PO_4$ ion. An abnormally high radioactivity of the heart as compared with the radioactivity of the heart of

the intact carp may be due to the individual difference in the cardiac muscular activity.

iii) Absorption from the head part and from the surface.

In Table 4 is given the deposition pattern of ^{32}P in the various tissues following the absorption by way of the head part or by way of the surface of the body including the digestive tract. To make these three separate experimental data comparable, the values for the radioactivity of the various tissues in these experiments were corrected for the difference in the radioactivity of the waters used. Data given in Table 4 indicate that the absorption of $^{32}P\ O_4$ ion took place similarly

Table 4. Absorption of $^{32}P\ O_4$ from head part and remainder part.
Water used; urethane, 0.25%; temperature, 13-13.5°C; radioactivity (cpm/cc), 3,660 for intact carp, 2,460 for exposure of head and 3,450 for exposure of remainder part.
All the Values given here are expressed as cpm/gr of fresh tissues and corrected for difference in radioactivity of the water used, viz., based on radioactivity at a level of 3,660 cpm/cc.

Site of absorption			Total body	Head part	Remainder part
Tissue \ Time of exposure (hr)			25	25	25
Blood			389	363	9.7
Gill			790	816	4.5
Scale			117	140	4.2
Caudal fin			189	198	25.4
Vertebra			25.8	27.9	2.0
Kidney			150	155	1.8
Liver			341	329	2.1
Heart			178	158	0.9
Spleen			78	87	0
Air bladder			56	46	2.7
Gall bladder			20.8	19.6	0
Gonad			♂ 15.5	♀ 6.7	♀ 0
Skin			52	41	2.5
Muscle			8.2	9.4	0
Gastrointestinal tract	Anterior	Tissue	161	191	1.3
		Content	327	3,887	4.9
	Posterior	Tissue	94	77	6.0
		Content	240	1,120	3.8
Body weight (g)			635	725	624

to ^{90}Sr (^{90}Y) largely by way of the head part, only a very small fraction being absorbed from the surface. That the gill is most likely responsible for the absorption in the head part is evidenced by the facts that there was always present in the gill the highest radioactivity among the tissues examined and nearly no absorption occurred from the digestive tract as cited above.

4. Summary

1. It has been shown that $^{32}PO_4$ ion in the water can be readily absorbed by the carp.

2. The absorbed ^{32}P has been found to exist in the gill, caudal fin and scale similarly to ^{90}Sr (^{90}Y) and ^{45}Ca. In contrast to ^{90}S (^{90}Y), the blood, heart, liver and kidney have been found high in ^{32}P.

3. It has been demonstrated that the absorption of $^{32}PO_4$ ion did not occur in the digestive tract nor from the surface of the body, but that it took place in the head part most likely by way of the gill.

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Distribution and Excretion of Intramuscularly Administered $^{32}P\ O_4$ by Carp

By

Tetuo Tomiyama , Kunio Kobayashi and Shinya Ishio

Distribution and Excretion of Intramuscularly Administered $^{32}P\ O_4$ by Carp

By

Tetuo Tomiyama*, Kunio Kobayashi** and Shinya Ishio***

1. Introduciton

In the preceding paper[1] evidence has been presented that $^{32}P\ O_4$ ion in water can be absorbed by the carp through the gill. The deposition pattern of the absorbed ^{32}P in the various tissues has also been determined. The present paper deals with the determination of the deposition pattern of the intramuscularly administered $^{32}P\ O_4$ ion and of its excretion channel.

2. Experimental Method

Two hours following the immersion in a 0.25% ethylurethane solution a carp weighing 580 gr was accommodated in the apparatus illustrated in a previous paper[2]. Both chambers were filled with a well-water containing 0.25% ethylurethane. The head chamber was supplied with oxygen at a rate of 100–150 cc per minute. Then, one cc of a neutralized $H_3{}^{32}P\ O_4$ solution having approximately 3×10^6 cpm was injected into the dorsal muscle just between two gummy membranes in order to insure the removal of $^{32}P\ O_4$ ion which might have diffused from an injection puncture. The experiment was carried out at 13–13.5°C. Twenty-five hours following the injection, the carp was removed from the apparatus and subjected to one-minute washing. The blood was sampled from the heart as usual. The blood and the various tissues were subject to the measurement of the radioactivity according to the procedure described previously[3]. The distribution of ^{32}P between the plasma and the corpuscles was determined in the same way as was reported previously[3].

In order to determine the excreted ^{32}P into two chambers of the apparatus, one-tenth total volume of the water in each chamber was removed and added with 1 cc of a 10% phosphoric acid solution. Following the addition of 1 cc concentrated ammonia, all the phosphate was precipitated by adding 5 cc of a 10% magnesium sulfate solution, filtered, dried, heated at 700°C. for 10 minutes and measured for the radioactivity.

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3. Results and Discussion

The distribution of ^{32}P in the various tissues is shown in Table 1, together with the ratio of the radioactivity of the tissues to that of the blood. It is seen that the distribution pattern in this case was nearly similar to that which was absorbed from the surrounding water. It is interesting to note that ^{32}P was considerably concentrated from the blood not only by the scale, caudal fin and gill, but also by kidney, liver, heart and spleen. The distribution of ^{32}P between the plasma and the corpuscles is shown in Table 2. Quite contrary to the distribution of

Table 1. Distribution of ^{32}P following intramuscular administration of its large dosis. ^{32}P administered, about 3×10^6 cpm per 580 gr; time of exposure, 25 hrs; temperature, 13–13.5°C; urethane, 0.25%.

Tissue	Radioactivity (cpm/gr)	Ratio to blood
Blood	1,907	1.00
Gill	5,267	2.76
Scale	13,667	7.17
Caudal fin	9,110	4.78
Vertebra	2,439	1.28
Kidney	12,794	6.71
Liver	9,996	5.24
Heart	9,036	4.74
Spleen	4,266	2.24
Air bladder	844	0.44
Gall bladder	608	0.32
Gonad ♂	427	0.22
Skin	779	0.41
Muscle	405	0.21
Digestive tract Anterior	1,351	0.71
Digestive tract Posterior	973	0.51
Contents of digestive tract Anterior	395	0.21
Contents of digestive tract Posterior	936	0.49

Table 2. ^{32}P in plasma and corpuscles.

	Plasma	Corpuscles
Radioactivity (cpm/gr)	1,380	2,539
Volume of plasma and corpuscles	63%	37%
Distribution of ^{32}P between plasma and corpuscles (cpm/gr of blood)	869	939

$^{90}Sr(^{90}Y)$ between them, ^{32}P expressed as specific activity was found in the plasma only half as much as in the corpuscles. It has been well known that phosphorus of the corpuscles is mostly in the organic form while that of the plasma is the inorganic phosphate. It can be concluded, accordingly, that the administered ^{32}P might be perfused by the blood and fairly rapidly incorporated in the corpuscles as the acid soluble phosphorus compound, leaving about 48% in the plasma as the inorganic phosphate.

The total excreted ^{32}P during 25 hours amounted to about 1% of the injected one. The ^{32}P excreted renally and extrarenally was found to be 28,296 and 2,796 cpm, respectively. High radioactivity of the gill made it conceivable to attribute the extrarenal excretion channel to the gill.

4. Summary

1. The distribution of ^{32}P in the various tissues and its excretion pattern have been studied following an intramuscular administration of a large dose of $^{32}P\ O_4$ ion to the carp.

2. It has been found that the deposition pattern in this case was qualitatively similar to that in case where $^{32}P\ O_4$ ion was absorbed from the surrounding water.

3. It has been clearly shown that about 10% of the total excreted ^{32}P was eliminated extrarenally.

4. About one-half of the ^{32}P in the blood was found in the corpuscles.

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生薑による魚臭の除去について

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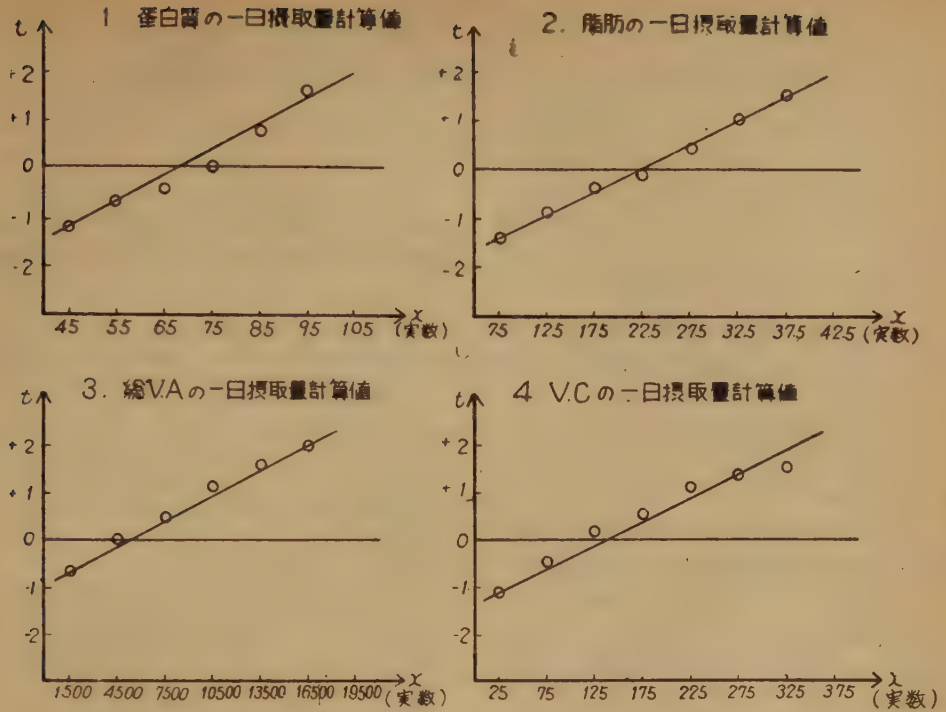

要　　旨

1950 年夏期（6～7 月）に実施された都市生活者の日常食中の $V.B_1$, $V.B_2$ 実測研究と同様の調査試験を 1952 年冬期 2～3 月，12 家族，36 試料につき行つた。今回は $V.B_1$, $V.B_2$ の他にプロ V.A を実測しそれ等の母平均の信頼限界を求めた結果，1 人 1 月摂取実測値は $V.B_1$ $775 \geqq m \geqq 665\gamma$，$V.B_2$ $652 \geqq m \geqq 488\gamma$，プロ V.A $5394 \geqq m \geqq 3434$ I.U. であつた。摂取 $V.B_1$ 実測値は 1950 年に於ける実測値より幾分低値をしめし，摂取 $V.B_2$ 量は多少増加の傾向を示したが著しい変動は認められなかつた。尚プロ V.A 実測値は一応標準所要量近くを示した。

尚之等実測値の計算値に対する割合を求めた結果，之等の母平均の信頼限界値は主食においては $V.B_1$ $55.1 \geqq m \geqq 42.3\%$, $V.B_2$ $73.3 \geqq m \geqq 59.9\%$, 副食に於いては $V.B_1$ $68.1 \geqq m \geqq 53.5\%$, $V.B_2$ $82.2 \geqq m \geqq 65.3\%$, プロ V.A $77.4 \geqq m \geqq 64.6\%$ なる割合を示した。

次に 1 人 1 日の摂取食品材料の重量より分析表にて栄養素の算出を行つた結果は蛋白質 $81.9 \geqq m \geqq 70.7$g，脂肪 $27.2 \geqq m \geqq 20.9$g，熱量平均 2015 Cal，総 V.A $7718 \geqq m \geqq 4761$ I.U. で 1950 年の夏期に於ける調査結果に比し総 V.A の損取計算値のみが著しく高値であつた。又 V.A の総 V.A に対する割合は 1 例を除いた 35 試料の平均で 3.9% という僅少に過ぎない結果を得た。

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生薑による魚臭の除去について

On the Deodorization of Fishiness by Adding a Ginger

（昭和 29 年 8 月 21 日受理）

富 安 行 雄　豊 水 正 道　高 橋 喜 久 雄
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調味並びに魚臭除去の目的で生薑を魚肉に添加することは日常行われている方法であるが，これに関する研究報告はないようである。著者等は実際に生薑の添加が魚臭除去に幾何程度有効であるか，及びその合理的な使用方法如何について検討した結果を報告する。

実 験 の 部

A. 実 験 方 法

ミンチにかけた鰯の全魚体を試料とし，これから得た溶液及び浸出液について実験を行つた。即ち溶液としては試料 10g を蒸気蒸溜して得た 50cc の初溜液を用い，浸出液としては試料 10g を各 25cc の蒸溜水で 2 回浸出して得た浸出液を遠心分離しその上澄液を分液して浮上油を除いたものを用いた。

脱臭効果は“卸し”ですつた生薑の一定量を共栓三角フラスコ中の被検液に添加し充分振盪して室温に 1 時間放置後，残存臭気を感覚によつて判定した。感覚では多少の個人差は避け得られないので常に 3 人以上の者で判定してその平均値を採ることにした。

B. 実 験 結 果

I. 生薑の魚臭除去効果　鰯の蒸気蒸溜液に生薑 1g を添加したところ第 1 表に示すごとく脱臭効果は pH 5.5～6.0 においては明かに認められたが，それよりも酸性側でもアルカリ性側でも次第に効果は減少した。この場

第 1 表　各種 pH における生薑の脱臭効果

pH	4.0	5.0	5.5	6.0	6.5	7.0	8.0
溶液(50cc)＋生薑(1g)	＋	±	−	−	±	＋	＋＋
対照（溶液）	＋＋＋	＋＋＋	＋＋＋	＋＋＋	＋＋＋	＋＋	＋＋

（＋，＋＋，＋＋＋ はそれぞれ魚臭の程度を表わし，± は痕跡的に魚臭のあることを，− は魚臭のないことを示す）

合生薑のにおいと魚臭とは全く異るものでありこの二者が同一溶液中に残存していても各々を嗅ぎ分け得るので生薑のにおいによつて魚臭が打消されたものでないことは明かに分る。浸出液でも魚臭除去の最適 pH は溶液と同様に pH 5.5～6.0 であつたので以後の実験では生薑の添加を pH 5.8 において行つた。

次に溶液と浸出液との脱臭に要する生薑の必要量の差異は第 2 表に示す通りであつて，浸出液では溶液の必要量の 5 倍を要した。然し乍ら煮沸湯煎中で 10 分間加熱した加熱浸出液では生薑の必要量は生浸出液におけるよりも減少した。

第 2 表　溶液及び浸出液の脱臭に要する生薑の必要量

生薑添加量(g)	溶液(50cc)	生薑添加量(g)	生浸出液(50cc)	加熱浸出液(50cc)
0.3	＋	1.0	＋＋	±
0.5	±	3.0	＋	−
1.0	−	5.0	−	−

II. 生薑による魚臭除去に対する蛋白質の阻害作用

前述のごとく浸出液は溶液よりも生薑により脱臭され難いが，その原因としては蒸溜による臭気成分の逸散，変化及び浸出液中における阻害物質の存在等が考えられる。浸出液は加熱によりある程度脱臭が容易になるので加熱の影響を更に検討した。即ち浸出液に生薑 2g を添加するに当つて加熱した浸出液に生薑を添加した場合と，生薑を添加して後加熱した場合とでは脱臭効果が異ることは第 3 表に示す通りであり，浸出液を加熱した後に添加した方が遙かに効果的であつた。臭気成分の逸散，変化等が浸出液を生薑の添加前又は後に加熱するこ

第 3 表　生薑の脱臭作用に及ぼす加熱の影響

生浸出液＋生薑	加熱浸出液＋生薑	生浸出液＋生薑⟶加熱
＋	−	＋

（浸出液 50cc，生薑 2g）

とにより特に変るとは考えられず浸出液中には生薑の脱臭作用に対する阻害物質が存在し，このものは加熱によつて不活性化されるものと考えるのが妥当であろう。

この点を明かにするために溶液にゼラチン及びオリー

ブ油（魚油は一種の臭気を有するので用いなかつた）を添加して生薑の脱臭作用に対する影響を試験した。その結果は第4表に示すようにゼラチンは脱臭作用を明かに阻害したが，オリーブ油には阻害作用は認められなかつた。そこで実際に鰯浸出液中に含有されている蛋白質が

第4表　生薑の脱臭作用に対するゼラチン及びオリーブ油添加の影響

添加量(g)	ゼラチン	オリーブ油
0.5	+	−
1.0	+	−
3.0	++	−

（溜液 50cc, 生薑 1g）

生薑の脱臭作用を阻害するや否やを確めるために蛋白沈澱剤を用いて試験した。即ち浸出液 50cc に 30% 醋酸鉛 1cc を添加した場合及び硫酸アンモン 15g を添加した場合における生薑の脱臭効果は第5表に示すようにこれ等沈澱剤を加えたのみでは魚臭は変化しなかつたが，沈澱剤を加えた後生薑 1g を添加すると完全に脱臭され

第5表　生薑の脱臭作用に対する蛋白沈澱剤の効果

処理方法 ＼ 沈澱剤	醋酸鉛	硫酸アンモン
浸出液＋沈澱剤	++	++
浸出液＋沈澱剤→＋生薑	−	−

（浸出液 50cc, 生薑 1g）

た。この外に沈澱剤として三塩化醋酸を用いたが特有の臭気のために効果は不明瞭であつた。

要　旨

魚臭を除去する目的で生薑を用いる場合には pH 5.5～6.0 で添加すれば最も有効である。又魚肉蛋白は生薑の脱臭作用を阻害するから加熱して変性させた後添加する方が効果的である。

（九州大学農学部附属水産実験所）

糸状菌の酵素生成に及ぼす Vitamin K_3 の影響について（第1報）

Studies on the effect of Vitamin K_3 for the produce of fungi enzyme

（昭和 29 年 9 月 3 日受理）

田 中 薫 樹　川 北 兵 藏
(Shigeki Tanaka)　(Hyozo Kawakita)

緒　　言

糸状菌類（殊に麹菌）には多くの酵素が含有されている事は周知のことである。殊にその生成する amylase, protease 等は醱酵工業上或は医薬品として主要な地位を占めている。我々は先に Vitamin K 類が[1)2)3)4)]食品保存料として相等著明な効果のあることを認めた。今回これ等 Vitamin K 類が微生物に及ぼす生理的影類を知らんとして先ず糸状菌（麹菌）の菌体量並びに酵素殊に Amylase 及び protease の生成に及ぼす影響について探索した。

一般に微生物の生育に阻害作用を有する元素及び化合物は一定の稀薄度に達すれば却つて生長を促進することが古くより知られている。Wooley は microbacterium paratuberculosis の増殖に phichiocol 及び Vitamin K_3 が必要であることを報告している[5)]。

我々は Vitamin K 類が或る濃度において微生物の生育を阻害する反面，他の或る濃度においては生長促進的に作用するのではなかろうか，又同時にその微生物の酵素生成にも何らかの作用を有するのではないかと考え，供試菌に Asp. oryzal を選び，Vitamin K_3 を添加培養し，その菌体量，amylase 及び protease 生成量について研究を行つた。今迄に得た成績に就て報告する。

実 験 方 法

1） **培地組成**　Czapeck-Dox 液体培地を基本としその窒素源及び炭素源を変えた 8 種類に就いて行つた。即ち炭素源は glucose を基本とし，他に lactose, sucrose, 及び soluble starch を各 40gr/l の割合で用いた。又窒素源は $NaNO_3$ を基とし他に $(NH_4)_2SO_4$, NH_4NO_3, urea, pepton, asparagine を選び夫々窒素量が 0.33gr/l になる様にした。pH を 6.0 に調整し各培地を 300ml 容三角フラスコに注入，常法に依り殺菌後使用した。

2） **供試菌**　当研究室保存の Asp. oryzal を用い Czapeck-Dox 寒天培地に 26°C, 72 時間培養したものより一白金耳を採り 10ml の無菌水に浮遊した液を各フラスコに 1ml 宛接種した。接種胞子数は約 5×10^5 個である。次で 26°～27°C で 10 日間静置培養を行つた。

3） **Vitamin K_3**　予め少量の 60%（vol/vol）の ethyl alcohol に溶解し，10^{-5}mol/l 単位で 6, 3, 1.5, 0.75, 0.375 になる様に各培養開始時に添加した。（以下単位は全て同 mol 数で示す）尚対照として Vitamin K_3 を添加しないもの及び Vitamin K_3 の溶解に用いた Alcohol 1×10^{-3}mol/l に就いても行つた。

4） **菌体量及び酵素液の調整**　培養物を濾過し母液と菌体に分け菌体は充分水洗後減圧デシケーター中で乾燥し菌体重量とした。次で石英砂を混じ乳鉢中でよく磨砕後重量の 20 倍量の 1% 食塩水で常温（20°C）で一夜（約 20 時間）抽出後濾過，濾液を酵素液とした。母液は pH を東洋濾紙 pH 試験紙で測定しそのまま酵素液とした。

5） **酵素力の測定**　Amylase は Willstäter—Schudel法[6)] に，protease は Fuld-Gross法[7)] に準じて測定した。即ち amylase は 2% soluble starch 20ml, McIlvaine 緩衝液 pH 5.5 3ml, 酵素液 1ml を 55°C, 1 時間反応後 n/10 Jod 溶液を必要量の 2 倍量程度加え，n/10 NaOH を滴下 15 分間放置した後 dil H_2SO_4 で酸性となし遊離した Jod を $Na_2S_2O_5$ 規定液で滴定 Jod の消費量を求めた。Protease は pH 6.0 の燐酸塩緩衝液 15%（vol%）を含む 0.1% Casein 2ml を 40°C 1 時間で消化する最少酵素量を求め母液及び菌体抽出液が消化する Casein mg 数で示した。

実 験 結 果

1） **菌体生成量**　炭素源として glucose を用い窒素源を異にした場合（以下単に “N 源を異にした場合” とする）（第 1 表）6mol では一般に菌体生成量少く阻止的に作用した。他の濃度では明瞭な作用は認められなかつたが urea を使用した場合 1.5mol で対照に比べ相当刺激的であつた。Asparagine 使用の場合は 0.375mol で刺激的に作用した。

窒素源として $NaNO_3$ を用い炭素源を異にした場合（以下単に “C 源を異にした場合” とする）（第 2 表）

Aureomycin によるイワシの鮮度保持*

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Aureomycin によるイワシの鮮度保持*

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Effectiveness of Aureomycin on Keeping Quality of Sardine

Tetuo Tomiyama, Minoru Nomura and Shunichi Kuroki

A considerable effectiveness in prolonging the storage life of the fish fillets has been obtained by a minute dip in minimal concentration of chlortetracycline[1,2]. However, no effectiveness for the shrimp has been observed at the concentraton used[2].

The present study shows that at least a 30-minute immersion in a 5% brine containing 10-20 ppm chlortetracycline is needed for a definite preservative effect on the sardine (Table 1). It has been found that the effectiveness on the keeping quality was increased a little with the increase in the concentration of the antibiotics (Table 2). It is of noteworthy that the brine in which the sardine was immersed was spoiled after one day, whereas the brine with chlortetracycline was judged still passable three days following a dip treatmemt of the sardine (Table 3). It has also been shown that a 10 ppm solution of chlortetracycline was able to treat the sardine at least by the same amount as its volume without any decrease in the effectiveness (Table 4). An overnight stowage of the sardine in coarsely cracked ice containing 10-20 ppm chlortetracycline followed by a storage at 4-8°C. without the ice has been found effective to prolong the storage life of the sardine as judged organoleptically or by the volatile base-N value (Table 5).

I 緒 言

Tarr 氏等[1]は 1948 年より 1954 年に亘り種々の抗生物質を用いて水産物の鮮度保持試験を行つている。その結果によると Aureomycin 或は Terramycin を使用した場合に最もよい効果がえられた。また 1954 年に Farber 氏[2]も 2 ppm の Aureomycin 液に 1 分間浸漬する操作によつてタラ肉の鮮度保持期間を無処理のものに比してかなり延長することに成功したが，エビそのまゝの形では同様の処理をしても効果は全くえられなかつたと報じている。

著者等は保蔵の最も困難なイワシの鮮度が Aureomycin によつて保持されれば，わが国の沿岸漁獲量の首位をしめる魚種であるから，漁村経営の上に稗益するところ極めて大きいと考えて本研究を行つた。その結果は適当な使用方法を実施すれば，従来のいずれの静菌剤によつても期待できなかつた程の著明な効果がえられたのでこゝに概要を報告する。

II 実 験 方 法

試料： 供試魚は市場でえられた大羽イワシを用いた。Aureomycin は純度 92% の粉末であつて，0.05% 液を原液とし，これを 5% NACI に所要濃度になるよう添加して用いた。

鮮度判定法： 従来種々の方法が提案されているが，一般に採用されている 30 mg % の揮発性塩基窒素を鮮度低下の限界とした。しかし自己消化の早いイワシの場合には，体内のデアミナーゼによつてアンモニアが生じるために 30 mg % とすることに多少の疑問がある。依て五感による判定をも参考のため記録した。揮発性塩基量の定量は富山・原田の高温減圧定量法[3]によつた。

処理方法： この方法としては魚種によりまた全魚体かフィレーかによつて色々な工夫がなさるべきである。イワシの場合には漁獲後に運搬船中で処理することが最もよいと考えて次の二つの方法について研究を

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進めた。即ち一つは薬品を含んだ氷水に浸漬処理する方法（A）と他は薬品氷で処理した後に冷蔵庫に貯蔵する方法（B）とである。（A）の方法に於て薬液に氷を入れることは実験中に濃度が変るので，これでは室温で処理を行った。即ち 5% NaCl 中に 40，20 及び 10 ppm の濃度にした Aureomycin 液 1 l. に 6 尾の大羽イワシを浸漬し，種々の時間経過後に引揚げ，液を切り，乾燥を防ぐためにパラフィン紙で包み，室温に放置した。以後毎日試料 2 尾について外観，臭気特に鰓部の変敗を観察し，次に背開きとし背部の肉の一定量を海砂と共に擂砕し，揮発性塩基の定量に供した。（B）の場合には Aureomycin を含む水道水を氷罐にて凍結し，次に砕氷にしたものを竹籠に並べたイワシの上に置いて冷蔵庫（4～8°C.）に一夜放置した。次に一尾宛パラフィン紙に包み，再び冷蔵庫に放置し，一定時間毎にその 2 尾宛をとり，（A）の場合と同様に五感検査及び揮発性塩基の定量に供した。

II 実験結果

1. Aureomycin 液に浸漬する時間の影響

Farber 氏[2]の採用した 1 分間浸漬条件の下に 10 ppm の液を用いて予備試験したところ，効果が極めて僅徴であったので，20 ppm 液を用いた場合の影響について吟味した。その結果は Table 1 に示すように，

Table 1. The effect of time of treatment on keeping quality of sardine[1].

Days stored at 18°C.	Quality \ Time of immersion	Immersed in 20 p.p.m. aureomycin hours 1/6	1/2	1	2	3	4	Control, 30-min. immersion in 5% brine
1[2]	Volatile base-N, mg.%	25	23	20	17	16	20	25
	Stale odour	—	—	—	—	—	—	±
2[3]	Volatile base-N, mg.%	54	41	44	40	39	39	78
	Stale odour	±	±	±	±	±	±	++
3[4]	Volatile base-N, mg.%	101	90	103	96	84	110	146
	Stale odour	++	±	±	±	±	±	+++

1) At start of the experiment, the sardine flesh contained 17 mg.% in volatile base-N.
2) All the samples were judged as fresh.
3) The control group : Eyes, fallen out ; skin, unclear. The aureomycin-treated group : keeping good quality.
4) All the samples were infected with fungi, though there was a marked difference in the development of the fungi between the control and the aureomycin-treated group.

10 分間浸漬した場合は効果が低いのに対し，30 分乃至 4 時間浸漬した場合はいずれも著明な効果を示し，揮発塩基窒素が 30 mg % に達した時間から判定すると，無処理の場合の鮮度保持期間を約 40% 延長した。なお五感検査の結果では，無処理群は 18°C. で放置 1 日後に既に僅か腐敗臭を示したのに対し，30 分間以上浸漬した処理群は 2 日後でも未だ鮮度良好であり，3 日後にも腐臭を示さず，内臓が僅かに干物類似臭を示したに過ぎなかつた。

2. Aureomycin 液の濃度の影響

Aureomycin 濃度を種々変した液に大羽イワシを 30 分間浸漬した後，18°C に放置した場合には Table 2 に示すような結果がえられた。即ち Aureomycin 10～40 ppm の範囲では 40 ppm が僅かに優れている

Table 2. The effect of aureomycin concentration on keeping quality of sardine[1].

Days stored at 18°C.	Quality \ Bacteriostatics conc.	Aureomycin p.p.m. 0	40	20	20[5]	10	Neo-furaskin p.p.m. 20
1[2]	Volatile base-N, mg.%	30	19	24	25	23	33
	Stale odour	±	—	—	—	—	±
2[3]	Volatile base-N, mg.%	78	49	57	54	53	86
	Stale odour	++	—	—	—	—	++
3[4]	Volatile base-N, mg.%	125	70	68	91	94	93
	Stale odour	+++	±	±	±	++	++

1) At start of the experiment, the sardine flesh contained 16 mg.% in the volatile base-N.
2) The control group, 5% brine treated : skin, unclear. The aureomycin-treated groups : skin, clear.
3) In all the samples, the flesh was observed to become soft. The neofuraskin-treated group : fungi developed.
4) The control group : skin, turbid. The neofuaskin-treated group : eyes, fallen out.
5) Vitamin C was added at a level of 0.05%.

ようであるが，いずれの場合も鮮度保持期間を約 50% 延長した。五感検査の結果では無処理群が 1 日で腐臭を示したのに対し，処理群は 3 日後に 10 ppm 液処理のものが腐臭を示したが，20～40 ppm 液処理のものは僅かに腐臭を示したに過ぎなかつた。20 ppm 液にビタミン C を 0.05% となるように添加し pH 4.5 とした液では揮発性塩基量からみると，ビタミン C を添加しない場合に比し良結果はみられなかつたが，五感検査の結果ではかなり良結果が認められた。これらの結果に対比して Neofuraskin 20 ppm 液で処理した群は揮発性塩基量並びに五感検査からみると，鮮度保持効果は殆ど認められなかつた。

次にイワシを浸漬した残液を 18°C. に放置しておくと，臭気，濁度，皮膜量について各区に著るしい差異がみられたので，一定量をとつて揮発塩基量を定量した結果は Table 3 に示すようであつた。この定量

Table 3 Deterioration of aureomycin solutions following immersion of the sardine[1].

Days stored at 18°C.	Quality \ Bacteriostatics conc.	Aureomycin p.p.m. 0	20	20[2]	10	Neofuraskin p.p.m. 20
0	Volatile base-N, mg.%	9				
	Stale odour	−	−	−	−	−
	Turbidity	±	−	−	−	−
1	Volatile base-N, mg.%	39	9	9	9	19
	Stale odour	+	−	−	−	−
	Turbidity	+	−	−	−	±
2	Volatile base-N, mg.%	59	19	13	20	39
	Stale odour	++	−	−	±	+
	Turbidity	++	−	−	±	+
	Film on surface	−				+
3	Volatile base-N, mg.%	70	20	15	35	57
	Stale odour	+++	±	−	+	+++
	Turbidity	+++	+	−	+	+++
	Film on surface	++	−	−	+	++

1) Six sardines weighing about 350 g. in total body weight were immersed in each experimental group.
2) Vitamin C was added at a level of 0.05 %.

結果及び五感検査からみると，処理魚体の場合と同様に 10 ppm 区の変敗が他区より多少早く，次に 20 ppm 区の順であり，40 ppm 区及びビタミン C 添加の 20 ppm 区は 3 日後にも全く変敗しなかつた。これらに対し Neofuraskin 20 ppm 区の防腐効果は遙かに微弱であつた。

3. 有効処理限度

Table 4. The effect of amount of the sardine treated by unit volume of a 10 p.p.m. aureomycin solution on storage life of the fish.

Dsays tored at 20°C.	Quality \ Amount of sardine treated / No. of treatment	1.1 kg. of the sardine in total per 1 l. of 10 p.p.m. aureomycin in 5% brine. #1[1]	#2[1]	#3[1]	#4[1]	#5[1]	Control, 5% brine only
1[2]	Volatile base-N, mg.%	19	19	27	—	20	23
	Stale odour	−	−	−	−	−	+
2[3]	Volatile base-N, mg.%	41	42	43	46	41	64
	Stale odour	−	−	−	−	−	+++
3[4]	Volatile base-N, mg.%	77	92	92	92	92	105
	Stale odour	+++	+++	+++	+++	+++	+++

1) Four sardines weighing about 200 g. in body weight were used for each treatment. At start of the experiment, the sardine flesh contained 15 mg. % in volatile base-N.
2) Appearance of the control group : gill, reddish brown ; viscera, extruded. That of the treated group : skin, clear ; eye, clear ; gill, red
3) The control group : gill, pale ; eye, unclear. The treated group : gill, red ; eye, clear.

一定量の 10 ppm Aureomycin 液により処理しえられるイワシの有効限度を知る目的で，氷冷した液 1 l に対し平均 54 g のイワシ 4 尾宛を 5 回順次にそれぞれ 30 分間浸漬し，次に液を除いた後に約 20°C の密閉罐中に放置し，各群毎に五感検査と筋肉の揮発性塩基の定量とを行つた。その結果は Table 4 に示すように，効果の減少する限度は求められなかつたが，液量と同量の魚体を浸漬した範囲では，浸漬回数によつて鮮度保持効果は全く低下しなかつた。

4. Aureomycin 氷による鮮度保持

実験方法の項に示したように 10 ppm 及び 20 ppm 含有氷で処理し，4～8°C. の冷蔵庫に放置した場合の揮発性塩基量の変化は Table 5 に示したようである。この定量値からみると，鮮度保持期間は約 30 % 延長している。しかし五感検査によると，無処理群が 4 日後に僅かに腐臭を感ぜられたのに，処理群では 10 日後に於ても腐臭が全く感ぜられなかつた。揮発性塩基窒素量からの判定で効果が少かつたのは恐らくは低温に於ても酵素作用による揮発性塩基の増加が起るためであろう。

Table 5. Effectiveness of aureomycin-containing ice on keeping quality of sardine[1].

Group	Quality \ Days stored at 18°C.	1	4	6	8	10
Control[2]	Volatile base-N, mg. %	18	25	29	74	92
	Stale odour	—	±	++	++	+++
20 ppm Aureomycin[3]	Volatile base-N, mg. %	17	18	22	34	62
	Stale odour	—	—	—	—	±
10 ppm Aureomycin[4]	Volatile base-N, mg. %	16	16	21	34	73
	Stale odour	—	—	—	—	±

1) At start of the experiment, the sardine flesh contained 16 mg. % volatile base-N.
2) After 1 day, gill was reddish brown ; after 6 days, eye was fallen out ; after 8 days, flesh became soft.
3) After 8 days, still passable ; after 9 days flesh was noticed to become soft.
4) Quite similar to the 20 p.p.m. aureomycin-treated sardine.

IV 考　　察

魚体の鮮度保持に対し現在迄に食品衛生法により許可された静菌物質は未だない。たゞニトロフラゾンが試験的に使用されているが，その効果について行われた多数の研究をみると，氷を併用することなしには効果は殆どないので，その使用には現在なお多くの問題を包蔵している。

各種抗生物質による魚肉或は魚体の鮮度保持の研究は最近のことであつて，その成果として Aureomycin が最も有効であるとされている。しかし魚体そのままでの鮮度保持には著効がみられていない。著者等はこの抗生物質の効果が魚体内への薬品の滲透量に支配されるものと予察して浸漬時間を変じてみたところ，30 分間以上浸漬することの極めて重要であることが明かとなつた。従つてイワシの場合には漁獲後直ちに輸送船に準備した Aureomycin 含有の氷水に浸漬したまゝで輸送すれば，魚体温度と浸漬液温度との差により，薬液が早く体内に滲透しまた輸送中に薬液が充分に滲透するために鮮度保持に有効なことは疑いない。また Aureomycin 氷によつて魚体に徐々に薬品を滲透させる方法が極めて有効であることが本研究で明らかになつたので，海上或は陸上輸送に使用することは極めて望ましい。

鮮度低下しやすいイワシに対して Aureomycin が示した鮮度保持効果は従来の静菌剤では全く期待できなかつた程の効果である。この点は本研究でえた Neofuraskin との比較結果からも明かである。しかし一般に食品の防腐に抗生物質を使用することは菌の耐性獲得が問題となる。Aureomycin は Penicillin や Streptomycin に比較すると，遥に菌の耐性を生じることが少いので，この点では安全で優秀な防腐剤といえるであろう。

なお本研究で用いた揮発性塩基量による判定は，無菌的に生じた揮発性塩基と腐敗により生じたものとを区別しえないので，論議の余地がある。従つてこの判定によると，一般に防腐効果は小さく現われる可能性があるので，更に揮発酸，トリメチールアミン，揮発性還元物等によつて再検討さるべきであることを附記する。

V 総　　括

1. オーレオマイシンによつてイワシの鮮度を保持するには，20 ppm の場合少くとも 30 分間以上浸漬せねばならない。

2. 30 分間浸漬の条件を採用すると，10～40 ppm の濃度範囲では浸漬後 18°C. に放置した場合，濃度の高い方が防腐効果に於て多少優つている程度であつた。揮発性塩基量により判定した鮮度保持期間は，約 40 % 延長した。これに対し 20 ppm の Neofuraskin は殆ど効果を示さなかつた。五感検査によると鮮度保持期間は無処理の約2倍に延長した。

3. Aureomycin 液にビタミン C を 0.05 % 添加すると，五感検査ではかなり良結果がえられた。

4. イワシ浸漬後の液を 18°C. に放置した場合には，無添加区のものは1日内に腐敗したが，添加区のものは少くとも3日間変敗しなかつた。この場合にビタミン C 添加の効果はかなり著明であつた。

5. Aureomycin 10 ppm 液 1 l. は少くともイワシ 1 kg の鮮度を保持する効果を示した。

6. 10 及び 20 ppm の Aureomycin 氷を用いてイワシを1日氷蔵した後，4～8°C に放置した場合には揮発性塩基量では鮮度保持期間が約 30% 延長した。五感検査によると，少くとも約 100 % は延長するようである。

本研究に使用した Aureomycin は日本レダリー及び武田薬品工業 KK より寄贈のものである。両社の協力に対し謝意を表する。

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Aureomycin によるウルメイワシの鮮度保持*

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Effectiveness of Chlorotetracycline (Aureomycin) on Keeping Quality of Pacific Round Herring, *Etrumeus micropus*

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In the previous papers[1)2)], it was shown that a considerable effectiveness in keeping quality of sardine and mackerel was obtained on treating with chlorotetracycline under appropriate conditions.

The present report deals with various possible procedures for the treatment of Pacific round herring with chlorotetracycline on a practical scale, aboard ship immediately after catching and/or on shore after landing.

The results indicate that the spoilage of fish was retarded markedly by storage aboard ship in sea water containing 10 p.p.m. of chlorotetracycline and ice containing 5 p.p.m. of chlorotetracycline or by storage in ice containing 5 p.p.m. of chlorotetracycline after landing without treatment with chlorotetracycline sea water on the boat. The effectiveness of chlorotetracycline in extending the storage life was tested under two storage conditions: 15 to 20°C. and −1 to 2°C. By judging from organoleptic examinations and values for total volatile bases and histamine, it was found that maximum prolongation of storage life over that of the control was 90% at 15–20°C. and 40% at −1 to 2°C.

緒　　言

富山・黒木及び野村[1)2)]はさきに市場よりえたイワシ及びサバに対し，オーレオマイシン（化学名 chlorotetracycline）を適当に使用すると鮮度保持効果が著名に認められることを報告し，またもし漁獲直後或は陸揚げ直後にオーレオマイシンで処理すれば，更に効果を増大することが期待されると推論した。

よつて昭和 30 年7 月末に熊本県牛深沖のイワシ巾着網で漁獲されたウルメイワシを用い，漁場・運搬船・或は冷蔵庫において実施しやすい実際的な処理方法に関して種々検討した。その結果適当と思われる方法が見出されたので報告する。

実　験　方　法

1. オーレオマイシン： 本研究には次の3種類を用いた。

a) オーレオマイシンA： 以下 A と略記す。純度 90.6% のものである。

b) Acronize B_5： 以下 B_5 と略記す。American Cyanamide Company, Fine Chemicals Division で氷添加用として調製したもので，懸濁安定剤を含み，オーレオマイシン含量は 16.5% である。

c) Acronize PD： 以下 P と略称する。海水溶解用として調製したもので，オーレオマイシン含量は 10% である。

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* 本研究は 1955 年 10 月長崎に於ける日本水産学会秋季大会で発表した。

浸漬用として用いた A 及び P 溶液はオーレオマイシン (chlorotetracycline) として 10ppm になる様に A 或は P を海水に溶解した。しかし実際には魚体と共に流入した海水のために，この濃度は一定に保持できなかつた。またオーレオマイシンはアルカリ性では抗菌力が低下するので，A 溶液は予め海水の pH が 6～7 になるように 1 規定酢酸を加えた後，1.5%A の 70% エタノール溶液を chlorotetracycline として 10ppm になるように海水に添加した。P は海水に溶解すれば pH が 6～7 になる様に予め調製されているものである。B_i 氷は chlorotetracycline の濃度が 5ppm になるように作つた。このためには Acronize B_i 4.5g を約 500cc の水に混和した後に氷罐内の 300lb の水中に添加し，10～60 分程度通気混和し，常法通り白氷にした。

2. **試料**： 供試魚は昭和 30 年 7 月 29 日に牛深より SE 約 10 哩の実習漁場において巾着網の 2 回の操業で獲た約 400 貫のウルメイワシで，体長 11～12cm，体重 18～20g のものであつた。ただし約 1～2% のマイワシが混入していた。

3. **処理方法**

船上処理： 18.4 トン，機関 65*HP* の昭徳丸母船で処理した。2 回の操業で漁獲した魚を Table 1 に示した方法で処理した。即ち 7 月 2 日の午前 4 時 30 分に漁獲した 60 箱（240 貫）のイワシを A 溶液に B 氷を混入した氷水（A+B 水氷）に浸漬し，また午前 5 時 10 分に漁獲した 40 箱のイワシを 2 分し，一部を P 溶液に B 氷を混入した水氷（P+B 水氷）に浸漬し，他の 1 部は海水に普通氷を混入した水氷に浸漬して対照区とした。なお 2～3 箱の魚を 4 斗樽に準備した A 溶液に普通氷を混入した水氷（A+I 水氷）に浸漬した。これらの処理は 3 つの魚艙と樽にそれぞれ予め準備した各水氷で行い，陸揚時まで浸漬した。各魚艙内の温度は加えた氷の量が一定しなかつたので多少の差はあつたが，A+B 水氷区 0.8～8°C，P+B 水氷区 3～6°C，A+I 水氷区 0.3～4°C，海水+普通氷区 0.5～5°C で比較的に近似していた。

Table 1. The methods of treatment of Pacific round herring by Aureomycin aboard and ashore.

Treatment on boat after capture[1]	Treatment after landing	The abbreviation of treatment
Immersion in sea water containing Aureomycin A[2)3)] plus Acronize B_i[2)4)] ice	held in Acronize B_i ice	A+B～B
	held in plain ice	A+B～I
	none	A+B
Immersion in sea water containing Acronize PD[2)3)] plus Acronize B_i ice	held in Acronize B_i ice	P+B～B
	held in plain ice	P+B～I
	none	P+B
Immersion in sea water containing Aureomycin A plus plain ice	held in plain ice	A+I～I
	none	A+I
Immersion in plain ice-sea water without Aureomycin	held in Acronize B_i ice	C～B
	held in plain ice	C～I
	30min.-immersion in Aureomycin A solution prior to icing without Aureomycin	C～A I
	30 min.-immersion in Aureomycin A solution only	C～A
	none	C

1) Fish were stored in ice-sea water with or without Aureomycin.
2) Three kinds of Aureomycin preparations were employed, namely, Aureomycin A, Acronize PD and Acronize B_i which contained 90.6%, 10% and 16.5% of chlorotetracycline, respectively.
3) The concentration of Aureomycin-containing solution was 10 p.p.m. as chlorotetracycline both in A and in PD.
4) The Aureomycin-containing ice were prepared with Acronize Biso as to result in 5 p.p.m. as chlorotetracycline.
5) The fish were placed in wooden boxes with about 3 kg. of appropriate ice per box containing about 15 kg. of fish.

陸揚後の処理： 船上で処理した漁獲物は7月29日午前6時30分に陸揚した。従つて漁獲から陸揚迄の時間はA+B水氷区が2時間，P+B水氷区，A+I水氷区及び対照区が約1時間30分であつた。陸揚後 Table 1 に示したように船上で処理した4区のいづれの区も夫々2分或いは3分し，1部にはB氷を，1部には普通氷をかけて氷蔵し，残部はそのまゝで放置した。但し普通水氷にのみ浸漬した対照区の一部は陸揚後 A 溶液に 30 分間浸漬した後，更に2分し，1部は普通氷で氷蔵し，他はそのまゝで放置した。いづれの区の氷蔵にも約 3kg の薬品氷或いは氷を用いた。

4. 放置条件： 上記のように処理した13区はいづれの区も一部を15～20°C の予冷室に各区別々に3列に積み重ねて放置し，13区の中，水揚後氷蔵した7区のみ一部を −1～+2°C の貯氷庫内に1列に並べて放置した。なお，汚水の混入を防ぐため1箱毎にビニールシートを置き，また全体をビニールシートで蓋つた。また積み重ねた場合に列及び上中下段の相違により鮮度低下に差を生ずると思われたので，列及び段を毎日入れかえ，なるべく条件が一定になるよう注意した。

5. 鮮度判定方法： 鮮度判定は官能判定と併行して筋肉中の揮発性塩基窒素及びヒスタミンの定量を行い，これらの値で客観的判定を行つた。揮発性塩基窒素は Conway の微量拡散定量法[3]により，ヒスタミンは門田，井上両氏の変改した cotton acid succinate 法[4]により定量した。これらの定量に供した試料は次のようにして調製した。即ち各区分より任意に20尾づつとり，外面を流水で洗滌した後，頭，内臓，脊骨等を除去して擂潰し，この肉 16g に 100cc の蒸溜水を加え，更に 7% 三塩化醋酸 50cc を混和し，その濾液を用いた。ヒスタミンの定量はこの濾液を中和して後に行つた。

結 果 及 び 考 察

1. 15～20°C に放置した場合

官能検査の結果は Table 2 に，揮発性塩基窒素 (V•B-N) の値は Table 3 に，ヒスタミンの値はヒスタミン塩酸塩として Table 4 に示した。

a) 船上におけるオーレオマイシン処理のみの場合の効果： Table 2 に示した官能検査の結果をみると，漁獲後腐敗臭を発するまでに要した時間は，水氷に浸漬した対照の C 区が 82 時間であつたのに対し，P+B 水氷に浸漬した区は 157 時間，A+I 水氷に浸漬した区は 132 時間，A+B 水氷に浸漬した区は 106 時間であつた。Table 3 に示した V•B-N の値から 30mg% に達するに要した時間を求めると，対照の C 区が 80 時間であつたのに対し，P+B 区は 143 時間，A+B 及び A+I 区は 100 時間であつた。対照区の鮮度保持期間を 1 とすると，鮮度保持延長効果はそれぞれ 1.8 倍，1.25 倍となる。また Table 4 をみると，HST-2HCl が 20mg% になる時間は，対照の C 区が約 90 時間であつたのに対し，A+B 区は全く差がなかつた。しかるに，P+B 区は 140 時間，A+I 区は 115 時間であつた。

以上の結果を綜合して判断すると，船上でオーレオマイシン水氷に浸漬し，陸揚後そのまゝ放置したものでは，P+B 水氷に浸漬した区が一番良く，次いで A+I 水氷に浸漬した区が良かつた。しかるに A+B 水氷に浸漬した区が A+I 区よりもやゝ劣り，且つ水氷のみに浸漬した対照の C 区に比べ余り効果がみられなかつたのは，A+B区が放置第1日目に，一番温度変動の激しい場所におかれたためであろう。この推測が正しいことは次に記した A+B 水氷に浸漬した後普通氷で氷蔵した A+B～I 区が，対照の水氷に浸漬後普通氷で氷蔵した C～I 区に比し著しく鮮度保持効果を示したことからも明かであろう。

b) 船上でオーレオマイシン処理し，陸揚後 B 氷或いは普通氷で氷蔵した場合の効果： Table 2 に示した官能検査の結果をみると，漁獲後腐敗臭を発する迄に要した時間は対照の水氷に浸漬し，陸揚後普通氷で氷蔵した区が 82 時間であつたのに対して，P+B の水氷に浸漬し，陸揚後 B 氷で氷蔵した区が最も鮮度良く 179 時間を要した。次いで A+B の水氷に浸漬した後 B 氷で氷蔵した区，A+B の水氷に浸漬した後普通氷で氷蔵した区，P+B の水氷に浸漬した後普通氷で氷蔵した区，水氷に浸漬後，B 氷で氷蔵した区及び A+I の水氷に浸漬後普通氷で氷蔵した区の順に良く 157 時間を要した。また，Table 3 に示した V•B-N の値から 30mg% に達するに要した時間を求めると，対照区が 80 時間であつたのに対し，P+B～B 区，P+B～I 区，A+B～B 区，C～B 区は 143 時間，A+B～I 区が 130 時間，A+I～I 区が 123 時間であつた。C～I 区の鮮度保持期間を 1 とすると，鮮度保持延長効果はそれぞれ 1.8 倍，1.62 倍，1.56

Table 2. Organoleptic change of Pacific round herring subjected to varying treatment with aureomycin.

The fish of 13 groups showed in Table 1 are stored at 15–20°C. The codes of the groups are shown in Table 1.

Hours of storage at 15–20°C	Organoleptic observations
8	A+B∼B, A+B∼I and A+B groups: Perfectly fresh. Rigor mortis just over. The other groups: Perfectly fresh. Still in rigor mortis
32	A+B group: Flesh slightly softer. Odor fresh. The other groups: Odor very fresh.
58	A+B∼B, P+B∼B and C∼B groups: Flesh firm. A+B∼I, P+B∼I, C∼AI, C∼A and C∼I groups: Flesh slightly softer. A+B group: Flesh soft. Surface fading. P+B group: Flesh firm. Surface slightly fading. A+I∼I, A+I and C groups: Flesh slightly softer. Surface slightly fading.
82	A+B∼B, P+B∼B and C∼B groups: Flesh soft. Eyes slightly opalescent and slightly sunken. A+B∼I, P+B∼I, P+B and C∼AI groups: Flesh soft. Eyes slightly opalescent. A+B group: Flesh very soft. Eyes slightly sunken. Surface noticeably fading. A+I∼I, A+I and C∼A groups: Flesh very soft. Eyes slightly opalescent. Surface fading. C∼I and C groups: Flesh very soft. Eyes slightly opalescent. Surface slightly fading. Odor stale.
106	A+B∼B, A+B∼I, P+B∼B, P+B∼I, P+B, A+I∼I, A+I and C∼B groups: Flesh very soft. A+B and C∼AI groups: Flesh very soft. Eyes opalescent, lost brilliancy and sunken. A particular odor different from putrid one. C∼I and C groups: Flesh very soft. Eyes opalescent, lost brilliancy and sunken. Surface fading. Odor putrid. C∼A group: Flesh very soft. Odor stale.
132	A+B∼B, A+B∼I, P+B∼B, P+B∼I, P+B, A+I∼I and C∼B groups: Flesh very soft. Surface fading. A+B group: Surface slimy. A particular odor different from putrid one. A+I group: Flesh very soft. Odor stale. C∼AI and C∼A groups: Flesh very soft. Eyes opalescent, lost brilliancy and sunken. Surface noticeably fading. Odor putrid. C∼I and C groups: Flesh very soft. Eyes opalescent, lost brilliancy and sunken. Odor very putrid.
157	A+B∼B, A+B∼I and A+B groups: Flesh very soft and very easily stripped from the bone. Surface slimy. Odor putrid. P+B group: Flesh very soft and very easily stripped from the bone. Odor still passable. P+B∼I, P+B, C∼AI, C∼A, A+I∼I, A+I and C∼B groups: Flesh very soft and easily stripped from the bone. Surface noticeably fading. Odor putrid.
179	A+B∼B, A+B∼I, P+B∼I, P+B, A+I∼I and C∼B groups: Odor very putrid. P+B group: Odor slightly putrid.

Table 3. The increase of volatile basic-N content (mg. %) during storage at 15–20°C.

Hours of storage after capture / Treatment*	Volatile basic-N mg. %							
	8	32	58	82	106	132	157	179
A+B~B		16.0	17.4	16.8	24.6	25.8	35.6	58.0
A+B~I		14.2	15.8	18.8	24.4	37.8	46.0	71.3
A+B	15.5	20.0	27.0	28.6	37.6	47.5	94.2	
P+B~B		18.0	15.6	19.2	23.4	27.0	41.2	58.1
P+B~I		15.0	17.8	19.2	26.6	33.0	45.8	63.5
P+B	15.0	19.2	21.8	23.2	25.4	36.4	57.8	64.8
A+I~I			17.2	20.6	25.6	31.2	46.2	94.4
A+I	16.1	22.2	25.8	25.0	29.4	44.2	67.6	
C~B		16.2	16.6	18.6	23.0	28.6	37.2	64.1
C~I		16.6	20.4	23.0	47.4	94.0		
C~AI		16.8	18.0	22.0	31.8	67.9	94.4	
C~A		22.2	23.6	27.4	40.2	59.0	88.0	
C	16.5	19.0	22.6	26.0	43.4	70.0		

* See Table 1.

Table 4. The increase of histamine content (mg. % histamine•2HCl) during storage at 15–20°C.

Hours of storage after capture / Treatment*	Histamine dihydrochloride, mg. %							
	8	32	58	82	106	132	157	179
A+B~B		6.8	6.8	10.9	10.9	10.9	21.3	25.9
A+B~I		6.8	10.9	10.9	10.9	10.9	27.1	41.4
A+B	0	6.8	17.0	15.0	22.5	25.9	37.3	
P+B~B		6.8	10.9	15.0	10.9	10.9	15.0	25.9
P+B~I		6.8	10.9	10.9	10.9	10.9	18.4	37.3
P+B	0	6.8	10.9	10.9	15.0	10.9	19.1	37.3
A+I~I		6.8		10.9	10.9	10.9	19.1	37.3
A+I	0	6.8	10.9	12.3	17.0	22.5	37.3	
C~B		6.8	10.9	10.9	12.3	19.1	19.1	45.4
C~I		6.8	10.9	17.0	37.3	45.3		
C~AI		6.8	15.0	15.0	22.5	38.6	53.1	
C~A		6.8	15.0	17.0	41.4	53.1	66.3	
C	0	6.8	17.0	17.0	22.5	38.6		

* See Table 1.

倍となる。また Table 4 に示した HST•2HCl の値から 20mg% に達するに要した時間を求めると，C~I 区が 90 時間であつたのに対し，P+B~B 区は 160 時間，C~B 区及び A+B~B 区は 150 時間，A+B~I 区，P+B~I 区及び A+I~I 区は 140 時間を要し，明らかにヒスタミン生成阻止効果を示した。

以上の結果を綜合して考察すると，船上でオーレオマイシン処理し，陸揚後 B 氷で氷蔵した区及び船上で水氷に氷漬し，陸揚後 B 氷で氷蔵した区はいづれの値もすぐれ．特に P+B の水氷に浸漬した後 B 氷で氷蔵した区の鮮度は最も良かつた。

船上でオーレオマイシン処理し，陸揚後普通氷を加えて保蔵した区（P+B~I 区，A+B~I 区，A+I~I 区）の鮮度保持効果は，船上でオーレオマイシン処理し，陸揚後 B 氷で氷蔵した区（P+B~B 区，A+B~B 区）の効果より僅かに劣つていた。かように，船上でオーレオマイシン処理し，陸揚後 B 氷で氷蔵し

た区（P+B～B 区，A+B～B 区）と普通氷で氷蔵した区（P+B～I 区，A+B～I 区，A+I～I 区）との間にそれ程鮮度保持効果の差がなかつたのは，漁獲量が少なかつたので魚がオーレオマイシン液に充分浸漬されたため，その後に加えた B 氷と普通氷との効果の差が余り現われなかつたものと推察される。他方，船上で水氷に浸漬し，陸揚後 B で氷蔵した区（C～B区）は，陸揚後普通氷で氷蔵した区（C～I 区）に比べて，鮮度保持に著効を示し，明らかに B 氷の効果を示した。

又，船上で A+B 水氷浸漬し，陸揚後普通氷で氷蔵した区（A+B～I 区）と，船上で A+I 水氷浸漬し，陸揚後普通氷で氷蔵した区（A+I～I 区）との間の鮮度保持効果の差は殆どみられなかつた。かように魚躰内のオーレオマイシン溶液に加えた B 氷と普通氷との効果の差がみられなかつたのは，漁獲量が少なかつたため魚に充分量のオーレオマイシンが滲透したためであろう。漁獲量が多い時には，水氷として普通氷を用いた場合よ，B 氷を用いた場合に比べて氷の融解によりオーレオマイシンの濃度が稀釈されるので鮮度保持効果が低下するであろう。

船上で P+B 水氷浸漬した後氷蔵したものと，A+B 水氷浸漬した後氷蔵したものの間には P と A との効果の差は殆どみられなかつた。

本実験において，船上で水氷に浸漬し，陸揚後 B 氷で氷蔵したのみの C～B 区が，A+B 水氷浸漬後 B 氷で氷蔵した A+B～B 区と同程度の鮮度保持効果を示しているのは，漁獲後陸揚迄に要した時間が短かつたためであろう。依つて漁獲後陸揚迄の時間が短い場合には，陸揚後 B 氷を加えるだけの簡単な処理でも鮮度保持期間をかなり延長できることが明らかとなつた。しかし，巾着網漁業において鮮度が最も問題とされるのは，漁獲して陸揚迄の間であるから，船上でオーレオマイシン処理することが望ましい。更に魚を長期保蔵する場合には，船上でオーレオマイシン処理し，陸揚後 B 氷で氷蔵する方法が最も理想的である。

c） 船上で水氷に浸漬し，陸揚後オーレオマイシン溶液に浸漬したものの効果： 船上で水氷に浸漬し，陸揚後 chlorotetracycline として 10ppm の A 溶液に 30 分間浸漬し，そのまゝ放置した区（C～A 区）及び陸揚後 A 溶液に 30 分浸漬した後更に普通氷で氷蔵した区（C～AI 区）は，船上で水氷に浸漬してそのまま放置した区（C 区）及び水氷に浸漬後普通氷で氷蔵した区（C～I 区）に比べて，V•B-N 及び HST•2HCl の値では鮮度保持効果は殆んどみられなかつたが，Table 2 より腐敗臭を発する迄の時間からみると 1.3～1.6 倍の効果があつた。

以上三項の試験は同時に行つたので，これらの結果を綜合して考察すると，船上において水氷で浸漬したのみで室温に放置した C 区と，船上において水氷に浸漬し，陸揚後普通氷を加え室温に放置した C～I 区との間には V•B-N，HST•2HCl 及び官能判定の結果から，放置初期には C～I 区が僅かに C 区より鮮度が良かつたが，C～I 区の氷が融解した後には，C～I 区と C 区との鮮度の間には殆んど差がなかつた。依つて，魚を氷蔵する場合，氷の使用量が少量で氷切れを生ずれば，鮮度保持を期待することは困難であることがわかつた。

船上において，PD+B 水氷に浸漬したのみの区（P+B 区）及び A+普通氷の水氷に浸漬したのみの区（A+I 区）は対照の C 区に比して明らかに鮮度保持効果が現れた。他方，船上において水氷に浸漬し，陸揚後 A 液に浸漬した区（C～A 区）は官能判定では僅かに鮮度保持効果が現れたが，V•B-N 及び HST•2HCl の値からは対照の C 区と殆んど差がなかつた。依つて，陸揚後にオーレオマイシンで処理するよりも，漁獲後すぐに船上においてオーレオマイシンで処理する事よ格段の鮮度保持効果を示すことが明らかである。

船上において，PD+B 水氷に浸漬したのみの区（P+B 区）と船上において PD+B 水氷に浸漬し，陸揚後普通氷で氷蔵した区（P+B～I 区）との鮮度保持効果は殆んど差がなかつたが，船上において PD+B 水氷に浸漬し，陸揚後 B 氷で，氷蔵した区(P+B～B 区)は P+B～I 区よりも大なる鮮度保持効果を示した。従つて，陸揚後普通氷で氷蔵するよりも B 氷で氷蔵することが鮮度保持効果の大きいことを示し，このことは C～I 区と C～B 区の間において顕著に現れている。

2. −1～2°C に放置した場合

Table 1 に示した 13 試験区の中，陸揚後 B 氷或いは普通氷で氷蔵した 7 区を −1～2°C の貯氷庫に放置した。Table 5 に示した V•B-N の値から 30mg% に達するに要した時間を求めると，船上で水氷に浸漬

Table 5. Effect of treatment by Aureomycin on keeping quality of Pacific round herring held at −1～2°C.

Days of storage after capture / Treatment*	Volatile basic-N mg. %						
	0[1]	2[2]	5[3]	9[4]	11[5]	14[6]	18[7]
A+B～B	14.0	14.6	15.8	15.8	20.3	17.3	33.8
A+B～I		14.8	15.0	15.0		18.5	38.3
P+B～B	15.0	15.8	16.0	16.6	18.4	21.0	35.8
P+B～I		15.2		15.4	20.0	22.5	49.3
A+I～I	15.0	16.2	16.6	17.0	20.3	21.4	50.0
C～B	14.0		14.4	16.2	20.3	23.5	44.0
C～I			14.8	15.6	21.5	49.3	75.8

* See Table 1.

1) A+B～B and A+B～I: Perfectly fresh. *rigor mortis* just over. The other groups: Perfectly fresh. Still in *rigor mortis*.
2) All the samples: Flesh firm. Very fresh.
3) P+B～B: Flesh slightly soft. Eyes slightly sunken. The other groups: Flesh slightly soft. Eyes opalescent and slightly sunken.
4) C～I: Flesh soft. Surface fading. General appearance stale. The other groups: Flesh soft. Surface fading.
5) C～I: Flesh very soft. Surface slimy. Odor putrid. The other groups: Flesh very soft. Surface slimy and stale.
6) C～I: Increase in slime. Odor very putrid. The other groups: Surface slimy. Odor putrid.
7) All the samples: Flesh very soft and easily stripped from the bone. Odor putrid. Among groups, C～I gave very putrid odor.

The temperature of storage room rose from −1～2°C/ to 2～8°C.

し，陸揚後普通氷で氷蔵した対照の C～I 区は 12 日であつたのに対し，船上で水氷に浸漬し，陸揚後 B 氷で氷蔵した C～B 区，船上で P+B 水氷に浸漬し，陸揚後普通氷で氷蔵した P+B～I 区及び船上で A+I 水氷に浸漬し，陸揚後普通氷で氷蔵した A+I～I区は約 15.5 日を要した。船上で A+B 水氷に浸漬し，陸揚後普通氷或いは B 氷で氷蔵した A+B～I 区及び A+B～B 区，また船上で P+B 水氷に浸漬し，陸揚後 B 氷で氷蔵した P+B～B 区は約 17 日を要した。各区の鮮度保持期間延長効果は C～I 区の鮮度保持日数を 1 とすると，C～B 区，P+B～I 区及び A+I～I 区は 1.3 倍，A+B～I 区，A+B～B 区及び P+B～B 区は 1.4 倍に相当する。他方，Table 5 に示した官能検査の結果をみると，腐敗臭を発するまでの時間は対照の C～I区が 10 日であつたのに対し C～B 区，A+B～I 区，A+B～B 区，A+I～I 区，P+B～I区及び P+B～B 区のオーレオマイシン処理区は 14～18 日で，1.4～1.8 倍の鮮度保持期間延長効果を示し，15～20°C に放置した場合と同じ結果がえられた。

Table 5 において V-B-N の値が漁獲後 14 日目から急増しているのは，この頃に貯氷庫の氷の出入が頻繁となり，放置温度が 2～8°C に上昇したので，鮮度低下が急激に進行したためであろう。もし放置温度が −1～2°C に維持されていれば，オーレオマイシン処理区は対照区に比してより以上の鮮度保持効果が現われたと思われる。

総　　括

イワシ巾着網で漁獲したウルメイワシを chlorotetracycline として 10ppm を含む Aureomycin A (A), Acronize PD (PD) 溶液及び chlorotetracycline として 5ppm を含む Acronize B_1 (B) 氷で処理し，−1～2°C 或いは 15～20°C に放置した場合の鮮度の低下を追跡し，オーレオマイシンの効果について検討した。

1. 船上でオーレオマイシン処理しただけのものについては，PD+B 氷の水氷に浸漬したものが一番よく，海水＋普通氷に浸漬しただけのものに比べて約 1.6 倍の鮮度保持効果を示した。

2. A+B 氷の水氷に浸漬後，B 氷或いは普通氷を加えたもの，PD+B 氷の水氷に浸漬後 B 氷或いは普

通氷を加えたもの或いは氷を加えないもの，A＋普通氷の水氷に浸漬後普通氷を加えたもの及び海水＋普通氷の水氷に浸漬後 B 氷を加えたものを 15～20°C に放置した場合，これらの試験区の鮮度保持期間延長効果は海水＋普通氷の水氷に浸漬した後普通氷で氷蔵したもの或いは浸漬後そのまゝ放置した対照の約 1.6～2.2 倍であつた。

3. 海水＋普通氷の水氷に浸漬し，陸揚後 chlorotetracycline として 10ppm の A 溶液に 30 分浸漬したものの鮮度保持効果は，対照に比して化学的検査では変らなかつたが，五感検査では多少みられた。

4. PD＋B 氷の水氷に浸漬後 B 氷或いは普通氷を加えて氷蔵したもの，A＋B 氷の水氷に浸漬後 B 氷或いは普通氷を加えて氷蔵したもの及び海水＋普通氷の水氷に浸漬後 B 氷で氷蔵したものを −1～2°C に放置した場合，これらの試験区は海水＋普通氷に浸漬後普通氷で氷蔵した対照の約 1.3～1.4 倍の鮮度保持期間延長効果を示した。

5. いづれの放置条件でも船上でオーレオマイシン水氷に浸漬し，陸揚後 B 氷を加えて氷蔵したものが最も優れた効果を示した。

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Aureomycin による底引漁獲物の鮮度保持*

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Effectiveness of Chlorotetracycline on Keeping Quality of Several Bottom Fish

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The authors (TOMIYAMA, KUROKI and NOMURA) have previously reported that the spoilage of sardine and mackerel was markedly retarded by Aureomycin (chlorotetracycline)[1-3]. The present report deals with prolongation of storage life of yellow croaker (*Pseudosciaena manchuria*), red sea bream (*Pagrosomus major*) and a species of sole (*Areliscus joyneri*) by treating with Aureomycin on board the trawler.

1) The freshness of yellow croaker after landing was the best in a group which had been subject to a 10-minute dip in Aureomycin (10 p. p. m.)-sea water immediately after capture, followed by holding in Aureomycin (5 p. p. m.)-ice on the boat for 13 days. The second best quality was found in a group which had been kept in Aureomycin (5 p. p. m.)-ice on the boat (Tables 1 and 2).

2) The combination of the dip in the Aureomycin-sea water and holding in the Aureomycin-ice on the boat gave a storage life at least 560 % longer than the control, i.e., a 10-day prolongation when yellow croaker were held in the corresponding ice after landing and left in a storage room at −1∼1°C. Under the identical condition of storage, the holding in the Aureomycin-ice on the boat without the dip treatment gave a storage life at least 360 % longer than the control, i.e., a 5-day prolongation (Tables 1 and 2). A certain extension of storage life was also observed when the fish was left at 21∼29°C. (Table 3).

3) Little effectiveness in keeping freshness was observed when red sea bream had been held in Aureomycin (5 p. p. m.)-ice on the boat for 20 days. The result might be due to the shortage of ice which was noticed after landing, because subsequent storage in Aureomycin (5 p. p. m.)-ice after landing showed a definite prolongation of storage-life compared to the control group held in plain ice (Tables 4 and 5).

4) Sole which had been held in Aureomycin (5 p. p. m.)-ice on the boat were transported from Fukuoka to Osaka, about three hundred miles by train and graded in the fish market of Osaka. It was noticed that the freshness of the fish held in Aureomycin (5 p. p. m.)-ice far exceeded the control held in plain ice particularly with respect to the body color and firmness of the flesh (Table 6).

緒 言

底引網漁業による魚獲量は，我国の年間総魚獲量の約 19 %** を占めている。また，底引網漁獲物は漁獲

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* 本研究は 1955 年 10 月長崎に於ける日本水産学会秋季大会で報告した。
** 昭和 29 年度農林水産統計による。

されてから食前に供されるまでには，2週間から約3週間を要する。従つて底引漁獲物の鮮度保持は，漁業経済及び国民栄養の上から極めて重要なことである。

富山・野村及び黒木[1]は，イワシの鮮度保持に対し，また富山・黒木及び野村[2]はサバの鮮度保持に対し Aureomycin が極めて有効なことを報告した。その後，更にイワシ巾着網漁船上での実際的試験[3]を行い，鮮度保持に最も有効な Aureomycin の使用方法を明かにした。依つて，これらの研究成果にもとづいて本研究では漁船上で漁獲物を直ちに Aureomycin 氷で氷蔵し，或は Aureomycin 液浸漬後 Aureomycin 氷で氷蔵し，次にこれら供試魚について，陸揚地及び消費地に於ける鮮度を，また陸揚後室温或いは冷蔵庫に放置した場合の鮮度の変化を測定し，底引網漁獲物の鮮度保持に対する Aureomycin の効果を検討した。

実　験　方　法

試料：　供試魚は 1955 年 8 月23日より 8 月 30 日までに，東支那海において村山水産K. K.の第8，第10広丸が底引網で獲たキングチ，マダイ，アカシタである。Aureomycin は海水溶解用として調製されている純度 10 %の Acronize PD，氷添加用として調製されている純度 16.5 %の Acronize Bi 及び純度 90.6 %の Aureomycin A の3種類を用いた。Aureomycin 氷は chlorotetracycline として 5 ppm の Acronize Bi 氷（Bi 氷と略記）或いは Aureomycin A 氷（A 氷と略記）及びそれに 30 ppm の Vitamin C を添加した Aureomycin A 氷（A+VC 氷と略記）を用いた。魚体浸漬用の Aureomycin 水氷は chlorotetracycline として 10 ppm の Acronize PD 溶液に Bi 氷を投入したもの（PD+Bi 水氷と略記）と，海水にその pH が 6～7 となるようにクエン酸を加えた後に，Aureomycin A を 1.5 %溶解した 50% Propylene glycol を海水の Aureomycin が 10 ppm となるように添加し，これに A 氷を投入したもの（AM−A+A 水氷と略記）を用いた。

処理方法：　底引船上で行つた Aureomycin 処理は次に示す2つの方法によつた。即ち1つは，漁獲後直ちに全魚体を PD+Bi 水氷或は AM-A+A 水氷に 10 分間浸漬した後，魚箱に並べ Bi 氷或いは A 氷を魚体の上にかけて，直ちに魚艙に貯蔵した浸漬−氷蔵法で，他は漁獲後箱に並べた魚体の上に A 氷，Bi 氷或いは A+V.C 氷のみをかけた氷蔵法である。なお，試験区はいづれも薬品氷が融解した場合に，薬品が魚体を洗うことが望ましいので，parchment paper を使用しなかつたが，普通氷のみで氷蔵した対照は，従来通り氷と魚体の間に parchment paper を用いた。

魚獲物は，陸揚後直ちに官能検査及び魚肉の揮発性塩基量を測定した。なお，一部を冷蔵庫（−1～+1°C）に他の一部を室温（21～29°C）に放置し，一定時間毎に官能検査及び揮発性塩基の定量に供した。また，残部は冷蔵貨車で大阪魚市場に送り，市場関係者の官能検査に供した。なお，冷蔵庫に放置した Aureomycin 処理区は −1～+1°C では殆んど変化しなかつたので，放置後9日目から 5～10°C の冷蔵庫に移して実験した。保蔵試験に供したもの及び市場に送つたものは，いづれも試験区には Aureomycin 氷を，対照には普通氷を陸揚時に増氷した。

鮮度判定法：　鮮度の判定は官能検査及び Conway 法[4]で測定した揮発性塩基量によつた。揮発性塩基の測定には，一定時間毎に任意に各区から5尾づつとり出し，その脊部肉をそれぞれ等量づつ切りとつて，mixer でよく細切混合し，これより 16 g 秤取し，100 cc の水と 7 %の三塩化醋酸 50 cc を加え，その濾液を供試した。

結　果　及　び　考　察

1.　キングチの鮮度に及ぼす Aureomycin の効果

1a. 陸揚時の鮮度：　1955 年8 月 29 日及び 30 日に漁獲したキングチの一部を PD+Bi 水氷，或いは AM-A+A 水氷に浸漬した後魚箱に腹建て並べ，Bi 氷或いは A 氷を魚体の上に被せた。他は Bi 氷或いは A 氷のみで氷蔵した。これらの Aureomycin 処理した魚を入れた箱は魚艙の中位部に積み，9 月 12 日に福岡魚市場に陸揚した。陸揚時の揮発性塩基の値及び官能検査の結果は，Table 1 及び 2 に示す通りである。これらをみると，PD+Bi 水氷に浸漬した後，Bi 氷で氷蔵したもの（以下 PD 浸漬—Bi 氷区と略記す）及び AM-A+A 水氷に浸漬した後，A 氷で氷蔵したもの（以下 A 浸漬—A 氷区と略記す）の鮮度は最も良く，それぞれ漁獲後 14 日或いは 13 日を経過しているにも拘らず，揮発性塩基は普通氷のみで氷蔵

Table 1. The increase of volatile basic-N content of flesh of the yellow croaker treated on boat with Aureomycin and held in Aureomycin ice in a cold room after landing.

The fish in group I were stored in the hold for 14 days on the boat after capture, while those in group II were stored for 13 days. The temperature in the hold was 1.3°C at the first period of the storage.

Treatment \ Days of storage after landing		Volatile basic-N, mg %												
		0	1	2	4	6	8	10	11	12	13	14	15	16
I	Held in plain ice	19.0	22.2	37.8	46.6	50.9	63.3	68.8	92.3					
	Held in Bi[2)3)] ice	15.2	27.0	37.8	41.4	56.8	58.5	66.6	80.6					
	Held in Bi ice after a 10 min-dip in PD[2)4)] solution	10.9	12.7	10.2	15.9	13.0	17.0	18.7	22.2	24.3	25.1	26.1	63.0	
II	Held in plain ice	29.3	31.7	30.9	37.9	55.8	62.2	74.5	75.6					
	Held in A[2)3)] ice	15.2	23.8		25.5	24.3	21.6	38.1	44.2	62.0	66.9	79.3		
	Held in A ice after a 10 min-dip in A[2)4)] solution	10.5	12.7	11.8	13.2	10.3	14.6	14.3	17.0	18.4	19.2	20.8	37.6	45.0

1) The fish were stored at −1∼1°C for 8 days after landing, and later were stored at 5∼10°C.
2) In this experiment three kinds of preparations Aureomycin were employed, namely, Aureomycin A (A), Acronize PD (PD) and Acronize Bi (Bi) containing 90.6 %, 10 % and 16.5 % of chlorotetracycline, respectively.
3) Ices containing Aureomycin were prepared by freezing water which was added with a freshly prepared alcoholic solution of A or suspension of Bi that was enough to make the concentration of chlorotetracycline 5 p. p. m.
4) The concentration of Aureomycin-solution was 10 p. p. m. as chlorotetracycline both in A and in PD solution.

Table 2. Organoleptic change of yellow croaker treated with Aureomycin.

Treatment / Days stored in cold room* after landing	Organoleptic observations					
	I*			II*		
	Held in plain ice	Held in Bi ice	Stored in Bi ice after a 10 min-dip in PD* solution	Held in plain ice	Held in A* ice	Held in A* ice after a 10 min-dip in A* solution
0	Flesh slightly softer. Eyes sunken and lost brilliancy. Surface slightly fading. Odor fresh.	Flesh slightly softer. Eyes lost brilliancy. Surface slightly fading. Odor fresh.	Flesh slightly firm. Eyes lost brilliancy. Surface brilliant. Odor fresh.	Flesh slightly softer. Eyes sunken and lost brilliancy. Surface fading. Odor stale.	Flesh slightly softer. Eyes brilliant and slightly sunken. Surface brilliant. Odor fresh.	Flesh slightly softer. Eyes brilliant. Surface brilliant. Odor fresh.
1	Flesh soft.	Flesh soft.	Flesh slightly soft.	Gill putrid.	Flesh slightly soft.	Flesh soft.
4	Gill putrid.	Eyes very sunken.	Flesh soft. Surface fading.	Gill putrid.	Eyes loft briliancy and sunken.	Eyes lost brilliancy. Odor fresh.
8	Flesh soft. Surface slimy. Odor putrid.	Flesh soft. Surface fading. Gill putrid.	Flesh soft. Eyes very sunken. Odor stale.	Eyes soft, sunken and opalescent. Surface slimy. Odor putrid.	Flesh soft. Eyes very sunken. Surface brilliant.	Flesh soft. Surface brilliant. Odor fresh.
11	Flesh soft. Eyes flowed out. Increase in slime. Odor putrid.	Flesh soft. Surface slimy. Odor putrid.	Flesh very soft. Eyes very sunken, and opalescent.	Flesh soft. Eyes flowed out. Increase in slime. Odor very putrid.	Flesh soft. Surface slightly slimy. Gill putrid.	Surface brilliant. Odor fresh.
13			Surface slimy. Odor stale.		Surface slimy. Odor putrid.	Flesh soft. Surface fading. Odor stale.
14			Odor slightly putrid.		Flesh very soft. Eyes flowed out. Increase in slime. Odor very putrid.	Flesh soft. Surface slightly slimy. Odor stale.
15			Flesh very soft. Increase in slime. Odor putrid.			Flesh very soft. Increase in slime. Odor putrid.

* See Table 1.

した対照区の 19 mg% 或いは 29 mg% に対し約 11 mg% にすぎず，また官能検査の結果も優れていた。次いで Bi 氷のみで氷蔵したもの（以下 Bi 氷区と略記す）及び A 氷のみで氷蔵したもの（以下 A 氷区と略記す）の鮮度が良く，揮発性塩基量はいづれも 15.2 mg% であり，官能検査の結果も優れていた。特に注目される点は Aureomycin で処理したものの腹部の体色が，いづれも鮮明な黄色を呈していたのに対し，対照のそれは褪色していた。また鰓の臭は，対照区は不快な腥臭がしていたのに対し，Aureomycin 区のものは腥臭が感じられなかつた。

1b. 冷蔵室に放置した場合の鮮度の変化： 供試キングチは 1955 年 9 月 12 日の陸揚から 8 日間は −1～1°C の冷蔵室に，9 日目以後は 5～10°C の冷蔵室に放置した。放置期間の揮発性塩基の変化及び官能検査の結果を Table 1 及び 2 に示した。普通氷で氷蔵した対照は，陸揚後約 12～33 時間目に鮮度低下の限界である揮発性塩基量 30 mg% に達したのに対し，Bi 氷区は約 37 時間目にこの値に達し，僅かに鮮度保持効果が認められた。他方，A 氷区，PD 浸漬—Bi 氷区及び A 浸漬—A 氷区では −1～1°C に 8 日間放置中の揮発性塩基量は，陸揚時の量と殆んど変らなかつた。依つて 5～10°C の冷蔵庫に移して，その変化を追跡したところ，A 氷区の揮発性塩基が 30 mg% に達したのは，試験開始後約 216 時間後で，PD 浸漬—Bi 氷区では約 336 時間後で，A 浸漬—A 氷区では約 350 時間後であつて，顕著な鮮度保持効果がみられた。

1c. 室温（21～29°C）に放置した場合の鮮度の変化： 陸揚後室温に放置した場合の揮発性塩基の変化及び官能検査の結果を Table 3 に示した。この場合も Aureomycin の水氷に浸漬した後，Aureomycin 氷で氷蔵したものの鮮度が最も良く，揮発性塩基が 30 mg % に達するまでに要した時間は，対照の約 0～12 時間に対し，PD 浸漬—Bi 氷区は約 28 時間，A 浸漬—A 氷区は約 30 時間であつた。次いで A 氷区が良く，約 16 時間後に揮発性塩基 30 mg % に達し，この場合の対照が既に陸揚時に鮮度低下の限界に達していたのに比すと，明らかに鮮度保持効果が認められた。Bi 氷区の鮮度は対照と殆んど差なく，効果が認められなかつた。

Table 3. Effect of treatment of Aureomycin on storage-life of Yellow croaker at 21～29°C.

Treatment		Days of storage after landing: Volatile basic-N, mg.% 0[1]	1[2]	2[3]
I	(a) Held in plain ice	19.0	45.2	97.5
	(b) Held in Bi* ice	15.2	47.6	101.0
	(c) Held in Bi ice after a 10 min-dip in PD* solution	10.9	25.9	99.1
II	(a) Held in plain ice	29.3	64.2	141.4
	(b) Held in A* ice	15.2	41.2	102.3
	(c) Held in A ice after a 10 min-dip in A solution	10.5	23.8	61.5

* See Table 1.

1) I (a) : Flesh slightly soft. Surface slightly fading. Eyes sunken and lost brilliancy. Odor fresh.
I (b) : Surface slightly fading. Eyes lost brilliancy. Odor passable.
I (c) : Flesh firm. Surface brilliant. Eyes lost brilliancy. Odor passable.
II (a) : Flesh slightly soft. Surface fading. Eyes sunken and lost brilliancy. Odor stale.
II (b) and II (c) : Flesh slightly soft. Surface brilliant. Eyes brilliant. Odor fresh.

2) I (a) : Flesh soft. Surface fading. Odor stale. Eyes very sunken.
I (b) and I (c) : Flesh soft. Surface brilliant. Eyes very sunken. Odor stale.
II (a) : Flesh soft. Eyes very sunken and opalescent. Odor putrid.
II (b) and II (c) : Flesh soft. Surface brilliant. Eyes very sunken. Odor stale.

3) I group : Surface slimy. Odor very putrid.
II group : Surface slimy. Odor very putrid.

以上の実験において，Bi 氷の効果が極めて僅少であつたことについては説明できないが，後記のタイの鮮度保持に Bi 氷が有効であつたことから考えると，この氷における Aureomycin の均一性については注

意を要するように思われる。しかし，いづれの場合においても Aureomycin 処理を行つたものは，腹部の体色は黄色を呈し，揮発性塩基の増加は対照に比して緩慢で，明らかに鮮度保持効果が認められた。Aureomycin 氷のみで氷蔵したものの腹部の黄色度及び他の外観は，浸漬氷蔵したものと殆んど変らなかつたが，揮発性塩基は浸漬氷蔵したものより急速に増加した。このことは Aureomycin 氷のみによる氷蔵は，魚体表面の汚染を防ぐのみであるが，これに反し Aureomycin 水氷に浸漬した後，Aureomycin 氷で氷蔵する方法は，体表面は勿論鰓その他体内からの汚染をも防止することを暗示している。

2. マダイ（小鯛，別名シバダイ）の鮮度に及ぼす Aureomycin の効果

2a. 陸揚時の鮮度： 1955 年 8 月 23 日漁獲したマダイを魚箱に脊建に並べ，魚体の上部に Aureomycin 氷を被せて 9 月 12 日まで魚艙の底部に積み貯蔵した。魚艙内の温度は操業の初期は 1.3°C であつたが，氷が少かつたため，操業の末期には魚艙の底部においていた試料の中には，氷切れのものがあつた。従つて，陸揚時のタイの鮮度は一般に悪く，また同じ区でも鮮度に多少のむらがあつたので，代表的なものを各区から一箱づつ選別して試験に供した。官能検査及び揮発性塩基の値は Table 4 に示す通りで，陸揚時の試験区の鮮度は，対照区のそれと殆んど差がなかつた。但し，Aureomycin A と vitamin C を入れた氷で氷蔵したもの（以下 A+V.C 氷区と略記す）の鮮度は対照区のそれより僅かに劣つていた。これは後に述べるように，氷切れのためと思われる。

Table 4. The effect of holding Aureomycin-ice after landing upon the storage-life at −1～1°C of red sea bream[1] which were held in plain ice or Aureomycin-ice on the boat.

No. of group	Days of storage after landing / Ice used on the boat and after landing	Volatile basic-N, mg.%					
		0[3]	1[4]	2[5]	4[6]	6[7]	8[8]
1	A*-ice	21.7	24.0	27.3	32.2	37.7	56.3
2	Bi*-ice	20.5	21.5	23.6	26.9	30.8	37.3
3	A+V.C[2] ice	29.8	29.5	30.6	31.6	29.8	59.0
4	Plain ice	21.6	23.9	26.5	32.7	63.9	68.3

* See Table 1.

1) Red sea bream employed in this experiment have been stored in the hold after capture for 20 days before landing. The temperature in the hold was 1.3°C.
2) A+V.C ice was prepared by freezing water which was added with enough amount of Aureomycin A and ascorbic acid to make chlorotetracycline 5 p.p.m. and ascorbic acid 30 p.p.m.
3) Group 1 and 3 : Flesh soft. Surface not brilliant. Odor somewhat putrid. Group 2. Flesh soft. Surface not brilliant. Odor putrid but stale. Group 4 : Flesh soft. Surface not brilliant. Odor putrid.
4) Groups 3 and 4 : Viscera extruded out.
5) Group 2 : Surface slightly slimy. The other groups : Surface slimy.
6) All the groups : Flesh soft. Odor putrid.
7) All the groups : Flesh soft. Increase in slime. Odor putrid.
8) Group 2 : Flesh very soft. Odor putrid. The other groups : Flesh very soft. Viscera extruded out. Odor very putrid.

2b. 冷蔵室に放置したタイの鮮度の変化： 陸揚後 9 月 20 日まで 8 日間 −1～1°C の冷蔵室に放置したタイの揮発性塩基の値及び官能検査の結果は，Table 4 に示してある。陸揚時は試験区と殆んど変らない鮮度を示した対照区は −1～1°C の低温で放置したにも拘らず，その鮮度は急激に低下し，揮発性塩基は約 72 時間で 30 mg % に達し，8 日後は 69 mg % を示した。一方，Aureomycin 氷で氷蔵した試験区の鮮度は，いづれも対照に比し緩慢に低下し，揮発性塩基量 30 mg % に達するに要した時間は，普通氷区が約 72 時間であつたのに対し，A 氷区が約 85 時間，B 氷区が約 120 時間，A+V.C 氷区が約 50 時間であつた。A+V.C 氷区は，水揚時の揮発性塩基が対照より多かつたので，対照より短時間で 30 mg % に達したが，その後の揮発性塩基の増加は緩慢で，8 日後の揮発性塩基は 59 mg % で，この場合は明らかに増氷により補われた Aureomycin の効果があつたものと思われる。従つて，陸揚時に A+V.C 氷区の鮮度が対照のそれより劣つていたのは，恐らく氷切れに原因したと推察される。

2c. 室温に放置したタイの鮮度の変化： 2a 項に記載したタイの一部を 21～29°C の室内に放置し，官能検査と魚肉の揮発性塩基を測定した。揮発性塩基の値と官能検査の結果は，Table 5 に示す通りで，冷蔵庫に放置した場合と全く同じ傾向を示した。即ち，対照の鮮度が急激に低下したのに対し，Aureomycin 氷を使用したものの鮮度は，いづれも緩慢に低下した。特に Bi 氷区の鮮度は最も良く，顕著な鮮度保持効果を示した。

Table 5. Effect of holding in Aureomycin-ice on storage-life of Red Sea bream** at 21～29°C.

No. of group	Days of storage after landing / Ice used	Volatile basic-N, mg.%		
		0[1]	1[2]	2[3]
1	A*-ice	21.7	42.8	100
2	Bi*-ice	20.5	29.4	100
3	A+V.C**-ice	29.8	49.2	100
4	Plain ice	21.6	80.1	100

* See Table 1. ** See Table 4.

1) Groups 1 and 3 : Flesh soft. Surface not brilliant. Odor somewhat putrid. Group 2 : Flesh soft. Surface not brilliant. Odor not putrid but stale. Group 4 : Flesh soft. Surface not brilliant. Odor putrid.
2) All the groups : Flesh very soft. Odor putrid. Blooded along the bone.
3) All the groups : Surface very slimy. Odor very putrid.

2d. 市場における鮮度： 大阪魚市場に冷蔵貨車で輸送し，9月 14 日市場関係者の五感検査に供した。その結果によると，普通魚のみで氷蔵した対照は，完全に腐敗していたか或いは非常に鮮度が低下していたのに対し，Aureomycin 氷で氷蔵したものは多少鮮度が優れていた。2b 項に記したように，氷が不足したので，魚艙の底部においたタイの内一部は氷切れのため鮮度にむらが出来た。従つて，氷切れの影響のない魚艙の中位部に積んだキングチ，アカシタ等にみられたような Aureomycin の顕著な鮮度保持効果は認められなかつた。

3. アカシタビラメの鮮度に及ぼす Aureomycin の効果

1955 年 8 月 30 日に漁獲したアカシタビラメを魚箱に並べ，一箱には A 氷を，他の一箱には普通氷を被せて 1.3°C の魚艙の中位部に積み氷蔵した。9 月 12 日に陸揚げし，直ちに数尾をとり出して官能検査及び揮発性塩基を測定した。陸揚時の揮発性塩基量は対照の 18.4 mg % に対し，A 氷区は 13.5 mg % であつた。残部は冷蔵貨車で大阪魚市場に直送し，市場関係者の官能検査に供した。その結果は，A 氷で氷蔵したアカシタビラメの体色は紅茶色で，肉がしまつていたのに対し，対照は茶褐色を呈し，肉は軟く，明らかな鮮度の差が観察された。大阪魚市場における官能検査の結果からも，Aureomycin A 氷が顕著な鮮度保持効果を示すことが明らかである。

結　　論

底引網でえたキングチ，タイ，アカシタビラメ等の鮮度保持に対する Aureomycin の効果について検討した。

1) 陸揚時のキングチの鮮度は chlorotetracycline として 10 ppm を含む Acronize PD 或いは Aureomycin A の水氷に 10 分間侵漬した後，chlorotetracycline として 5 ppm を含む Acronize Bi 氷或いは Aureomycin A 氷で氷蔵したものが最も良く，次いで Bi 氷或いは A 氷のみで氷蔵したものの鮮度が良かつた。

2) 船上で処理したキングチを陸揚後に冷蔵室（−1～1°C）に放置した場合には，Aureomycin 処理をせず，普通氷で氷蔵した対照区は 1～4 日で初期腐敗に達するのに対し，Aureomycin 水氷で処理後 Aureomycin 氷に貯蔵した区は，少くとも 14 日を要した。Aureomycin 氷のみで貯蔵した区は，約 9 日を要した。また室温（21～29°C）に放置した場合にも，同様の傾向が認められた。

3) 漁獲後 20 日を経過したタイの陸揚時の鮮度について，官能検査及び揮発性塩基を測定したところ，

Aureomycin 氷で氷蔵したものの水揚時の鮮度は，氷不足のため普通氷で氷蔵したものの鮮度と差がなかつた。

4) Aureomycin 氷で氷蔵したタイは，陸揚時対照と近似の鮮度を示したが，−1～1°C 或いは 21～29 °C に放置したところ，いづれの場合も Aureomycin 氷で氷蔵したものの鮮度は，普通氷で氷蔵したものより緩慢に低下し，明らかに Aureomycin の鮮度保持効果が認められた。

5) Aureomycin 氷で氷蔵したアカシタビラメの鮮度は，普通氷で氷蔵したものの鮮度より遙かに優れていた。

終りに，本研究の遂行に多大の御支援を戴いた武田薬品工業株式会社，日本レダリー及び村山水産株式会社に深謝の意を表す。

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食品の鮮度測定法に関する研究—Ⅷ.
蒲鉾の新鮮度測定法*

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食品の鮮度測定法に関する研究——Ⅷ. 蒲鉾の新鮮度測定法*

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Studies on the Method for Testing the Spoilage of Food——Ⅷ. A New Turbidimetric Method for Determination of Freshness of Cooked Fish-paste "Kamaboko".

Tetuo Tomiyama, Yasuo Yone and Norisuke Sugawara

During the spoilage of the cooked fish-paste, it is invariably observed that its surface is gradually decomposed by the development of various micro-organisms. It follows that, on washing the surface with water by using a brush, a turbid washing will be resulted, its turbidity being proportional to the degree of development of the micro-organisms.

Data presented in this paper have revealed that freshness of cooked fish-paste can readily be estimated by determining the turbidity of the washing which is made with 250 cc. water by employing a brush of soft hair. The turbidity measurment has been carried out by the electro-photometer with a filter of 470 mμ. As is shown in Fig. 4, a marked increase in the value for the turbidity during the spoilage has been found to be in good conformity with a marked increase in the catalase activity of the washing which was already reported as an index of incipient spoilage.

Ⅰ. 緒　　言

著者等は第7報[1]に於て，蒲鉾の鮮度低下中に於ける蒲鉾表面のカタラーゼ作用力の変化は官能検査成績とよく平行し，迅速且つ容易に測定できるので，この方法は蒲鉾の鮮度測定に適当であることを提唱した。その研究中に洗液の濁度がまた蒲鉾表面の汚染度と平行することに気付いたので，洗液の濁度と鮮度との関係を検討すれば，カタラーゼ作用力による方法よりも優る簡易測定法が決定できるであろうと考え，本研究に着手した。種々吟味した結果，実用的に便利な新測定法を決定できたので，以下その詳細を報告する。

Ⅱ. 鮮 度 測 定 法

筆を用い，一本の蒲鉾の表面を 250 cc の蒸溜水或いは水道水で充分に洗滌する。この洗液をよく攪拌した後，その一部を比濁用試験管にとる。再びよく攪拌し，3～5 分後 470 mμ のフィルターを用いて光電比色計** でその濁度（吸光度）を測定する。その値が 0.3 以下であれば，食品として安全と見做す。

Ⅲ. 実験結果及び考察

1. 濁度測定用試料採取法の吟味

著者等[1]は既にカタラーゼ作用力による蒲鉾の鮮度測定の場合に，洗滌に使用するブラッシュの種類により洗液に混入する肉片の大きさ及び量が異り，また洗滌の程度に差があることを認めた。依て先づゴムブラッシュ或は筆を使用した場合に洗液の濁度が静置中にどの程度変動するかにつき吟味した。その結果は Fig. 1 に示すように，筆を用いた場合の濁度は殆んど変動しなかつたのに対し，ゴムブラッシュを用いた場合には時間的な減少が著しかつた。次に洗滌回数によつて洗液の濁度がどの程度減少するかを検討してみた。そ

1955 年6月 13 日受理

* 本研究の大要は 1952 年 12 月　佐世保に於ける日本水産学会九州支部大会で発表した。

** 東京光電式度計5型

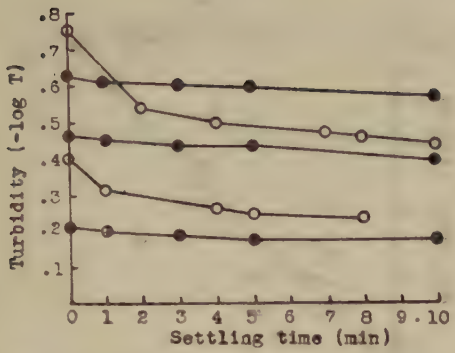

Fig. 1. Change in turbidity on standing the washing. Gummy brush ○, soft hair brush ●.

の結果は Table 1 にみられるように，第1回洗液の濁度に対する第2回洗液の濁度の割合は，鮮度がよい程大きいが，変敗したものではこの割合は小さいので，鮮度表示の大きな誤差にはならない。しかも第3回洗液の濁度は第2回のものと殆ど変らないので，鮮度低下に伴う表面のみの変化は，第1回洗滌に現われるとみてよい。以上のような吟味から，筆による洗滌が適当であること，洗滌は一回だけで充分であることがわかつた。

2. 洗液の稀釈による濁度の変化

洗液を稀釈することによつて，濁度がどの程度減少するかを Fig. 2 の値から検討すると，光電比色計の読みが0.9以下の場合は比例関係が認められたが，0.9 以上の場合は直線関係を示さなかつた。従つて0.9以上の値を示す場合は0.9以下を示すように適当に稀釈して測定し，測定値に稀釈度を乗じた値を用うれば良い。

Table 1. Change in turbidity of washings which are made by washing with different kinds of brush and by repeating washing.

Kinds of brush / Samples / No. of washing	Gummy brush				Soft brush		
	A	B	C	D	A	B	C
1st washing	0.071	0.17	0.89	0.620	0.080	0.201	1.070
2 nd washing	0.035	0.08	0.35	0.252	0.038	0.090	0.204
3 rd washing				0.233			

All the values are expressed as $-\log T$ obtained by electro-photometer.

3. 蒲鉾の鮮度低下と濁度との関係

鮮度測定の規準を求めるため，鮮度低下の初期と洗液の濁度との関係をみた。即ち無糖板付蒲鉾を密閉罐内に収容して 29°C に放置し，逐次，外観，カタラーゼ作用力[1]の変化及び濁度の変化を追跡した。その結果は Table 2 及び Fig. 3 に示すように 50 cc の水を用いた洗液の濁度が 1.1 に達した頃より鮮度低下が初まり，1/5 稀釈のものの濁度が 0.3，カタラーゼ作用力が 40 mm Hg 附近になつて細菌が散見され，しかもこの値附近から濁度及びカタラーゼ作用力が急激に上昇した。Fig. 2 の結果を考慮すると，1/5 稀釈のものの値を用いるのが適当と考えられる。依つて無糖板付蒲鉾を 25°C に放置し，250 cc の水で洗滌して同様の実験を行つた。その結果は Table 3 に示すように，同じ傾向が認められた。更に多数の無糖板付蒲鉾を用いて同様の実験を行い，洗液の濁度とカタラーゼ作用力との関係をみた結果は Fig. 4 に示す通りで，濁度0.3のときのカタラーゼ作用力は約 45 mm Hg であつた。この結果は変敗初期のカタラーゼ作用力は 50 mm Hg であるとの前報[1]の結果と一致している。

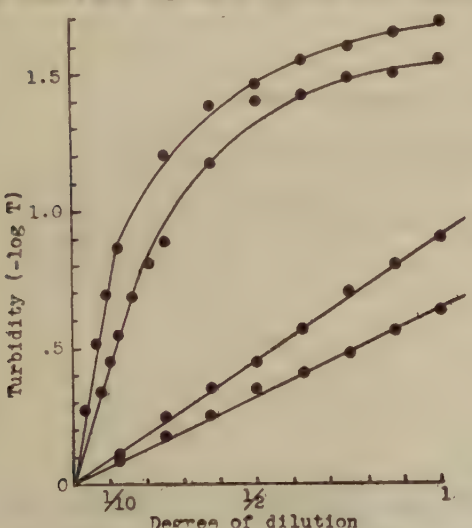

Fig. 2. Relationship between the turbidity and dilution of the washing.

Ⅳ. 総　括

1. 蒲鉾表面の汚染度を知るには，蒲鉾の全表面を 250 cc の水を用い，柔い筆で充分洗滌してえら

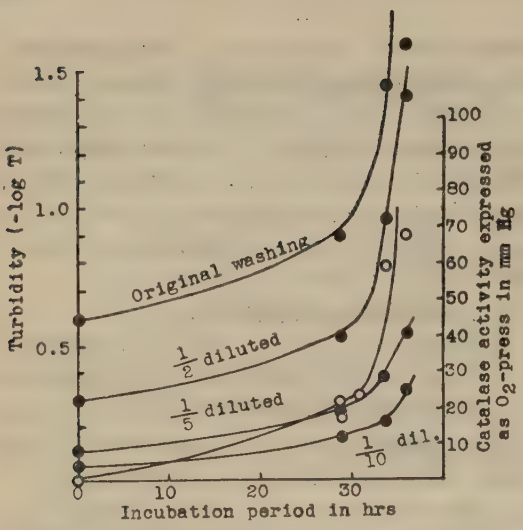

Fig. 3. Change in turbidity and catalase activity during the incubation.
Turbidity ●, catalase activity ○.

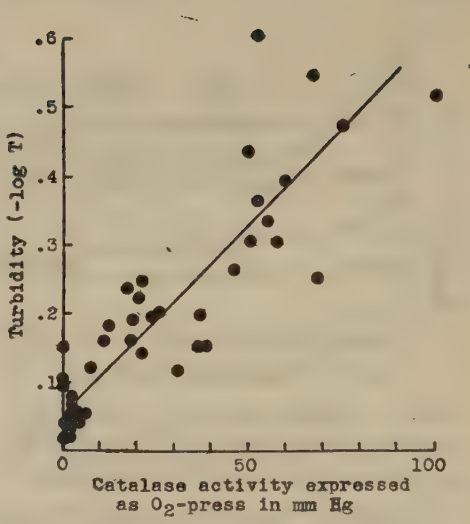

Fig. 4. Relation between turbidity and catalase activity of the washing of surface of the cooked fish-paste.

Table 2. Change in turbidity and catalase activity during the incubation of cooked fish-paste.

Time on incubation at 29°C.	Turbidity				Catalase activity 1/5 dil..	"Neto"**		Decay odor	Good ○ or no × as food
	No dil.*	1/2 dil.	1/5 dil.	1/10 dil.		All over surface	Scattering		
0	0.597	0.280	0.110	0.052	0	−	−	−	○
	0.620		0.107			−	−	−	○
29	0.899	0.511	0.235	0.150	17	−	±	±	○
	0.93	0.519	0.245	0.163	21	−	±	±	○
31	0.94	0.518	0.198	0.115	24	−	−	±	○
	1.41	0.85	0.360	0.205	52	−	+	+	×
34	1.55	1.08	0.512	0.285	103	−	++	+	×
	1.45	0.96	0.390	0.210	59	−	+	+	×
36	1.60	1.41	0.542	0.320	67	−	+	+	×

* Washed with 50 cc. water. ** Development of mucilagenous bacteria.

Table 3. Relation between initial stage of spoilage and turbidity of the washing.

Time on incubation at 25°C.	Catalase activity at 5 mins	Turbidity -log T	"Neto"		Decay odor	Fungi	Good ○ or no × as food
			All over surface	Scattering			
0	0	0.04	−	−	−	−	○
17	1.5	0.08	−	−	−	−	○
34	22	0.22	−	±	−	−	?
44	57	0.30	−	+	±	−	×
61	241	1.07	+		+	−	×

れる懸濁液について，その濁度を測定するのが適当である。

2. 無糖板付蒲鉾を 29°C 及び 25°C の密閉罐内に放置した場合，食品の価値を失う点と表面洗液の濁度の急昇点とは一致した。この点は洗液の濁度が 0.3 の時である。また濁度 0.3 のときのカタラーゼ作用力は約 45 mm Hg であつて，変敗の初期を示す値 50 mm Hg とほゞ一致した。

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煉製品の鮮度保持に関する研究 I

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紫外線，赤外線，包装及び塗布した防腐剤の効果*

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I. 緒 言

蒲鉾の鮮度保持の目的を達する手段として，1）包装による二次的汚染の防止，2）紫外線及び赤外線による表面の微生物の減滅，3）防腐剤による内部変敗の防止の三つをとりあげて，著者等は1950年秋より研究を開始し，セロファン包装の優秀なこと，赤外線及び紫外線による処理は極めて有効なこと，これらの物理的手段は防腐剤のみによる化学的方法よりもむしろ優ることを認めて，1951年の日本水産学会臨時大会に於てその概要を報告した．本研究の報告後に，煉製品の鮮度保持に関する綜合研究が発足したが，多数の研究の結論として著者等の試みた包装が鮮度保持に極めて優秀であることを再確認している.[1] 他方紫外線照射の効果については，鉄本氏等[2]の追試結果は著者等の結果と一致していないが，これはその照射方法の差に原因するようである．ここにその後の研究をも追加し取纏め報告し，今後の研究の発展に寄与したい．

II. 実 験 方 法

a） 試料の調製及び放置条件：試験に用いた蒲鉾は無糖板付のもので，大略擂砕肉90～94%，馬鈴薯澱粉9～5%，食塩1%其他味の素，サッカリン少量より成る業者の製造したものである．試料の保持は飽和湿度とした大形のブリキ缶に20～30本の試料を入れ，蓋をして放置した．

b） 防腐剤の種類及び塗布方法：供試したものは BHC（純度99%"リンデン"），過酸化水素水，イソアミールガレート IAG，パラオキシ安息香酸ブチルエステル POB，ニトロソフェノール NP，マレイン酸 MA，グアノフラシン GF，ビタミンK_3，メチールオレアート，オレイン酸，カプリン酸，スクアレン，第四級アミン QA（AB），QA（BB）（日本油脂研究所製造の日産カチオン AB 及び BB で有効成分30%のもの），ソルビタンモノオレアート（日本油脂研究所製）である．これらの防腐剤はブラッシュを以て表面に塗布し，なお対照区には無処理のもの及び試験区と同一湿度となるよう溶媒を塗布したものを用いた．

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* 本研究の大要は1951年11月及び1952年4月に日本水産学会年会で発表した．

c） 紫外線及び赤外線照射方法：紫外線の照射にはマツダ或はシマダの 15W の低圧殺菌灯を用い，30cm の距離で10分或いは15分間上面或いは全面を照射した．赤外線の照射にはマツダの 500W S型のものを用い，30 cm の距離から上面或いは全面を10分或いは15分間照射した．15分間全面照射の場合は上面5分間，側面を3分間宛，前後面を2分間宛照射した．

d） 包装方法：セロファンは予め紫外灯で照射して殺菌したものを用い，全面を完全に包むように注意した．

e） 鮮度の判定：鮮度は主として官能検査によつたが，なお試験中試料の揮発性塩基を富山，原田[3]の高温減圧法で定量した．但し従来一般に揮発性塩基測定の場合には，摺砕した全蒲鉾から試料を採取しているが，このような方法で採取した試料から得た揮発性塩基の変化は官能検査と一致しないので，著者等は試料の表層の 1cm 厚の肉をとつて測定した．

III. 結 果 及 び 考 察

1．各種防腐剤の効果及びセロファン包装の効果

A．無包装の場合に於ける防腐剤の効果

各種防腐剤の水溶液，エタノール溶液或いはソープレスソープ乳化液の 10cc をそれぞれ一本の蒲鉾の表面に塗布した．但し BHC は懸濁液を用いた．

Table 1 の官能検査の結果をみると，無処理の対照区は24時間後から僅かに異臭を発し

Table 1. Effectiveness of several chemicals to keeping quality of "Kamaboko" without casing.

Time on incubation at 22～29°C (hr)		24			48				72			
Chemicals \ Quality		"Neto"	Fungi	Odor [1]	"Neto"	Fungi	Odor	V.B.N mg % [2]	"Neto"	Fungi	Odor	V.B.N mg %
Ethanol	[3]	—	—	±	++	—	+	28.8	++	—	+	34.8
BHC	[4]	—	—	—	+	—	±	17.6	++	+	+	29.1
IAG	[5]	—	—	—	++	—	+	19.4	++	—	+	30.4
POB	[6]	—	—	—	±	—	±	21.1	++	—	+	35.6
H_2O_2	[7]	—	—	—	—	—	—	9.4	—	+	±	16.4

1) Colonies of mucilagenous bacteria. 2) Volatile basic nitrogen of fish cake were measured by Tomiyama and Harada's method.[3] 3) 20% ethyl alcohol. 4) 99% BHC "Linden", 0.02% BHC suspension in 20% ethanol. 5) 0.02% Iso-amyl gallate in 20% ethanol solution. 6) 0.02% butyl ester p-oxybenzoic acid in 20% ethanol solution. 7) 1% H_2O_2. 3～7) 10cc of each preservative were smeared on the surface of a fish cake, followed by being kept in a closed vessel.

初め，48時間後は微生物が認められたのに対し，1％過酸化水素水は最も有効で，72時間後にカビが発生した．72時間後の揮発性塩基は無処理の対照区の 34.8 mg％ に対し 16.4 mg％ を示したにすぎなかつた．なお BHC，IAG，POB 等は本実験の範囲では殆ど効果が認められなかつた．

脂肪酸類の防腐効果は Table 2 に示すように，過酸化物価の高いオレイン酸が僅かに防腐効果を示したが，他の脂肪酸類（メチールオレアート，カプリン酸，スクアレン）は無効であつた．

Table 2. Effectiveness of fatty acids to keeping quality of "Kamaboko" without casing.

Time on incubation at 25℃ (hr)	24			48		
Quality / Chemicals[1]	"Neto"	Fungi	Odor	"Neto"	Fungi	Odor
None	—	—	—	##	—	+
0.02％ Soapless soap *	—	—	—	+	—	+
0.02％ Methyl oleate	±	—	—	##	—	+
0.02％ Methyl oleate **	±	—	—	+	—	+
0.02％ Oleic acid	—	—	—	##	—	+
0.02％ Oleic acid†	—	—	—	±	—	—
2.0 ％ Oleic acid†	—	—	—	±	—	—
0.02％ Capric acid	—	—	—	##	—	+
0.02％ Squalene	—	—	—	+	—	+
0.02％ Squalene §	—	—	—	##	—	+

* "Mikesoap"　** † High peroxide value　§ Squalene deteriorated.

1) All samples were emulsfied in 0.02％ soapless soap solution.

第四級アミン類の効果は Table 3 に示すように，QA（AB）及び QA（BB）はいづ

Table 3. Effectiveness of quaternary ammonium compounds to keeping of "Kamaboko" without casing.

Time on incubation at 25℃ (hr)	24			48		
Quality / Chemicals	"Neto"	Fungi	Odor	"Neto"	Fungi	Odor
None	+	—	+	##	±	+
QA(AB)* 1 ％	—	—	—	+	—	+
QA(AB)* 0.2％	+	—	+	##	±	+
QA(BB)* 1 ％	—	—	—	±	±	±
QA(BB)* 0.2％	±	—	±	+	±	+
0.2% QA(BB) + 0.2% Oleic acid	±	—	±	+	±	+
1% H_2O_2 + 0.2% Oleic acid	—	—	—	±	±	±

* Quaternary ammonium compounds, 30% purity

れも1％の濃度で有効であつた．これらの試料中の有効成分含量は30％であるから有望な防腐剤と思われる．また前実験で効果があつた1％過酸化水素水と0.2％オレイン酸の乳化液は有効であつたが，単独効果と比較すると相乗的効果は認められない．Sorbitan mono oleate，過酸化水素水及びオレイン酸の併用効果は Table 4 に示すように，期待した効果は得られなかつた．

Table 4. Effect of combination of hydrogen peroxide with surfactant and oleic acid to keeping quality of "Kamaboko".

Time on incubation at 26°C (hr)	72			96			120		
Chemicals \ Quality	"Neto"	Fungi	Odor	"Neto"	Fungi	Odor	"Neto"	Fungi	Odor
None	+	+	+	++	+	+	++	++	+
1% H_2O_2 + 1% Sorbitan mono oleate	−	+	−	−	++	+	−	++	+
1% H_2O_2 + 1% Sorbitan mono oleate + 0.2% Oleic acid	−	+	−	−	++	+	−	++	+

B．セロファン包装の場合に於ける防腐剤の効果

防腐試験は次に示すように2回行つた．試験Ⅰでは防腐剤を蒲鉾の表面に塗布した後，直ちにセロファンで完全に包装し，24～30°C の室に放置した．その結果は Table 5 に示すように，試験区はいづれも無包装の対照区より微生物が多発し，揮発性塩基の値も大きかつた．またエタノールを塗布して包装した対照区が無包装の対照区より鮮度保持効果が劣つているが，それに反し包装のみした対照区は多少効果が認められたことから考えると，試験区が無包装に比し劣つた原因は，蒲鉾の表面が細菌の発育に適した水分を保持したためと思われる．依て試験Ⅱでは予め蒲鉾に赤外線を照射して表面を加温した後，防腐剤を塗布し，数分放置後包装した．その結果は防腐剤を塗布した試験区では POB，H_2O_2，H_2O_2＋MA，GF の効果が僅かに認められたにすぎない．無包装の場合に於ける効果と比較してみると，包装と防腐剤を併用した場合に於けるカビの発生防止効果は包装による二次的汚染の防止効果によるものと思われる．

2．赤外線，防腐剤及びセロファン包装の効果

A．無包装の場合に於ける効果

蒲鉾の表面に防腐剤を塗布した後，赤外線で蒲鉾の上面を10分間照射し，密閉缶に入れて22～29°C の室に放置した．官能検査及び揮発性塩基の値は Table 6 に示すように，エタノールを塗布した対照区は24時間後に異臭を発し初め，48時間後には細菌が多発して腐敗臭を生じた．また揮発性塩基は48時間後に 28.8 mg％，120時間後は 67.7 mg％であつた．しかし赤外線のみ照射したものは48時間後に蒲鉾の側面に僅か細菌が発生し，72時間後には側面に発生した細菌が繁殖したのみで上面には全く発生しなかつた．揮発性塩基は72時間後に 12.4 mg％，120時間後は 29 mg％ で顕著な鮮度保持効果が認められ

Table 5. Effectiveness of casing to keeping quality of "Kamaboko".

Experiments	Time on incubation at 24～30°C (hr) / Casing / Chemicals	With cover○ Without×*	24 "Neto"	24 Fungi	48 "Neto"	48 Fungi	48 V.B.N mg %	72 "Neto"	72 Fungi	72 V.B.N mg %
I**	None	×	±	−	+	±	10.6	##	##	17.8
	None	○	−	−	+	−	8.3	+	±	14.4
	Ethanol 1)	○	−	−	##	−	22.4	##	±	28.2
	BHC 2)	○	−	−	##	−	19.9	##	−	24.2
	IAG 3)	○	−	−	##	−	21.5	##	±	26.5
	POB 4)	○	−	−	##	−	16.1	##	−	20.8
	NP 5)	○	−	−	##	−	18.4	##	±	22.2
	H_2O_2 6)	○	−	−	±	##	6.2	##	##	28.5
II***	None	×	−	−	−	+				
	None	○	−	−	±	±				
	BHC 2)	○	−	−	##	−				
	POB 4)	○	−	−	±	−				
	H_2O_2 6)	○	−	−	±	−				
	H_2O_2+MA 7)	○	−	−	±	−				
	GF 8)	○	−	−	±	−				
	VK_3 9)	○	−	−	±	±				

* Cover just after the application.　** Cover after drying up applied chemicals.　1) 2) 3) 4) 6) See Table 1. 5) 8) 9) 0.02% chemicals in 20% ethanol solution.　7) 1% H_2O_2 was adjusted to pH 5 with maleic acid solution.

Table 6. Effect of combination of infrared ray and preservatives.

Time on incubation at 22～29°C (hr)		24			48				72				96			120
Treatment / Quality		"Neto"	Fungi	Odor	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %
Ethanol*		−	−	±	##	−	+	28.8	##	−	+	34.8	##	##	+	67.7
Irradiation by infrared ray**	None	−	−	−	±	−	−	10.2	+	−	+	12.4	##	##	+	29.0
	BHC*	−	−	−	+	−	+	17.0	+	−	+	26.8	##	+	+	35.9
	IAG*	−	−	−	+	−	+	14.4	+	−	+	23.9	##	##	+	45.5
	POB*	−	−	−	+	−	+	25.9	+	−	+	28.0	##	+	+	45.6
	H_2O_2*	−	−	−	−	−	−	11.0	±	−	−	16.2	##	+	+	32.7

* See Table 1.　** The upper part of "Kamaboko" was subjected to a 10 minute irradiation with a 500-W infrared ray lamp at a 30cm distance.

た．赤外線と防腐剤とを併用した場合は，過酸化水素水を塗布したものが，72時間後に蒲鉾の側面に細菌が僅か発生し，揮発塩基は 16.2 mg% で多少効果があつたが，120時間後には官能検査の値は赤外線のみを照射したものに比して殆んど差なく，また揮発性塩基は 32.7 mg% となり差が認められなかつた．他の防腐剤を塗布したものはエタノールを塗布した対照区に比すと多少効果があつたが，赤外線のみを照射したものよりは劣つていたので防腐剤の効果は認められない．赤外線を照射したものはすべて先ず側面に細菌が発生し，4日後に上面に細菌が発生し始めたことは，赤外線照射方法に改善すべき点のあるのを示している．

B．セロファン包装した場合の効果

防腐剤を蒲鉾の表面に塗布した後，直ちにセロファンで完全に包装し，赤外線で蒲鉾の上面を15分間照射した．なお赤外線のみを照射した区は試験開始日のみ照射したものと毎日1回照射したものを設けた．他の試験区は全て試験開始日のみ照射した．防腐試験の結果は Table 7 に示すように，包装して試験開始日のみ赤外線を照射したものは包装した対照区と比較すると差がなかつた．しかし無包装或いは包装して毎日赤外線を照射したものはいづれも無処理及び包装した対照区より優れ，48時間後に蒲鉾の側面に細菌が僅か繁殖した程度であつた．なお96時間後の揮発性塩基は無処理の対照区の 20.6 mg% に対し，無包装で毎日赤外線を照射したものは 18.9 mg%，包装して毎日赤外線を照射したものは 15.7 mg% で赤外線による鮮度保持効果が認められた．また毎日赤外線を照射した2区の中

Table 7. Effect of combination of infrared ray, preservatives and casing.

Time on incubation at 24～30°C (hr)		24		48			72			96
Treatment \ Casing	With cover ○ Without ×	"Neto"	Fungi	"Neto"	Fungi	V.B.N mg %	"Neto"	Fungi	V.B.N mg %	V.B.N mg %
None	×	±	−	+	±	10.6	##	##	17.8	20.6
None	○	−	−	+	−	8.3	+	±	14.4	19.0
Ethanol*	○	−	−	##	−	22.4	##	±	28.2	31.4
Irradiation by infrared ray** None†	×	−	−	±	−		+	−	15.2	18.9
None†	○	−	−	±	−		±	−		15.7
None	○	−	−	+	±		+	±	17.7	20.6
BHC*	○	−	−	+	−		+	±	22.1	27.8
IAG*	○	−	−	+	−		+	±	15.4	19.3
POB*	○	−	−	+	−		##	±	16.5	22.7
NP*	○	−	−	+	−		+	±	16.3	20.4
H_2O_2*	○	−	−	±	−		±	+	13.2	24.4

* See Table 1 and 5. ** The upper part of "Kamaboko" was subjected to a 15 minute irradiation with a 500-W infrared ray lamp at a 30 cm distance. "Kamaboko" was covered with ce llophane just after the application of chemicals, and then irradiated. † Daily irradiation.

で包装したものは無包装のものより細菌の繁殖程度が多少おそく，揮発性塩基も少なかつたのは包装による二次的汚染の防止効果と思われる．防腐剤を併用した場合は防腐剤を溶解するために用いた溶媒を塗布して包装した対照区に比して，いづれの試験区も効果が認められた．この効果は防腐剤による効果であるか，赤外線による効果であるかはこの実験のみでは明かでない．しかし Table 5 に示したように，防腐剤塗布後直ちに包装したものは無処理の対照区より悪かつたことから考えると，恐らく防腐剤塗布後直ちに包装し赤外線を照射したものの効果は赤外線による効果であろう．

3．紫外線，防腐剤及びセロファン包装の効果

A．無包装の場合に於ける効果

蒲鉾の表面に防腐剤を塗布したのち紫外線を蒲鉾の上面に毎日 10 分間照射した．Table 8 をみると，エタノールを塗布した対照区は 24 時間後に異臭を発し，48 時間後には細菌が多発し，120 時間後の揮発性塩基は 67.7 mg% であつた．これに対し紫外線のみを照射した区は 96 時間後に数点のカビのコロニーが認められたにすぎず，120 時間後の揮発塩基は 16.2 mg% で顕著な鮮度保持効果を示した．しかし防腐剤と紫外線とを併用したものは紫外線のみを照射した区と大差なく，防腐剤による効果は全く認められなかつた．

Table 8. Effect of combination of ultraviolet ray and preservatives.

Time on incubation at 22～29°C (hr)		24			48				72				96			120
Treatment \ Quality		"Neto"	Fungi	Odor	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %
Ethanol*		−	−	±	++	−	+	28.8	++	−	+	34.8	++	++	+	67.7
Irradiation by ultraviolet ray**	None	−	−	−	−	−	−	5.8	−	−	−	9.0	−	±	−	16.2
	BHC*	−	−	−	−	−	−	8.8	−	−	−	9.4	−	+	−	21.8
	IAG*	−	−	−	−	−	−	7.8	−	−	−	7.8	−	±	−	19.6
	POB*	−	−	−	−	−	−	7.1	−	−	−	8.4	±	±	±	17.6
	H_2O_2*	−	−	−	−	−	−	6.1	−	−	−	10.1	−	±	−	16.8

* See Table 1.　　** The upper part of "Kamaboko" was subjected to a 10 minute irradiation daily with a 15-W ultraviolet ray lamp at a 30cm distance.

B．セロファン包装した場合に於ける効果

本試験は 2 回行つた．試験 I では蒲鉾の表面に防腐剤を塗布した後，直ちにセロファンで完全に包装し，紫外線を蒲鉾の上面に毎日 10 分間照射してみた．官能検査及び揮発性塩基の値は Table 9 に示すように，無処理の対照区は 24 時間後に細菌が僅かに発生し，48 時間後には微生物が多発した．またエタノールを塗布した後包装した対照区は 48 時間後に微生物が多発した．紫外線のみを照射したものは 48 時間後に細菌が蒲鉾の側面に僅か発生したが，その後の細菌の繁殖速度は緩慢であつた．なお 120 時間後の揮発性塩基は無処理の対照区の 29.8 mg%，包装した対照区の 50.0 mg% に対し，紫外線のみ照射したも

のは 13.0 mg% で顕著な効果が認められた．防腐剤と紫外線とを併用したものの中で，過酸化水素水を塗布したものは72時間後に蒲鉾の前後面にカビが発生したが，細菌は120時間後に僅か発生した程度であり，120時間後の揮発性塩基は 6.9 mg% で最も有効であつた．しかし他の防腐剤の効果は認められなかつた．

Table 9. Effect of combination of ultraviolet ray, preservatives and casing.

Experiments	Time on incubation at 24～30°C (hr) / Casing / Treatment	With cover ○ Without ×	24	48			72			96			120		
			"Neto"	"Neto"	Fungi	V.B.N mg %	"Neto"	Fungi	V.B.N mg %	"Neto"	Fungi	V.B.N mg %	"Neto"	Fungi	V.B.N mg %
I	None	×	±	+	±	10.6	##	##	17.8			20.6	##	##	29.8
	Ethanol*	○	−	##	−	22.4	##	±	28.2			31.4	##	##	50.0
	Irradiated upper part only † None	○	−	±	−		±	−	7.9			8.3	##	##	13.0
	Irradiated upper part only † BHC*	○	−	±	−		+	−				15.7	##	+	15.6
	Irradiated upper part only † IAG*	○	−	±	−		±	±				13.7	##	+	16.0
	Irradiated upper part only † POB*	○	−	±	−		+	±				11.0	##	+	15.1
	Irradiated upper part only † NP*	○	−	±	−		+	±				10.9	##	+	12.7
	Irradiated upper part only † H_2O_2*	○	−	−	−		−	+				9.0	±	##	6.9
II	None	×	−	−	+		−	##		+	##				
	None	○	−	±	±		+	+		##	##				
	Irradiated all over the surface § None	○	−	−	−		±	−		±	±				
	Irradiated all over the surface § BHC*	○	−	−	−		−	−		±	−				
	Irradiated all over the surface § POB*	○	−	−	−		−	−		±	−				
	Irradiated all over the surface § H_2O_2*	○	−	−	−		−	−		−	−				
	Irradiated all over the surface § H_2O_2 +MA*	○	−	−	−		−	−		±	−				
	Irradiated all over the surface § GF*	○	−	−	−		−	−		±	−				
	Irradiated all over the surface § VK_3*	○	−	−	−		±	−		+	−				

* See Table 1 and 5. † A 10 minute irradiation. § Irradiated 5 minute for the upper part, 3 minute for both long sides and 2 minute for both short sides. The irradiation was carried out daily with a 15-W ultraviolet ray lamp at a 30cm distance.

以上の試験に於て防腐剤を塗布した後，直ちに包装した場合は常にその効果が対照区より多少劣ること，また赤外線或いは紫外線を蒲鉾の上面にのみ照射した場合は，先ず蒲鉾の側面に微生物が発生することから，試験Ⅱでは蒲鉾を赤外線で2～3分間照射し加温した後，防腐剤を塗布し，数分後セロファンで完全に包装して紫外線を蒲鉾の全面に毎日 15 分間照射した．官能検査の結果は Table 9 に示すように，無処理及び包装した対照区は 48 時間後に微生物が多発したが，これに対し包装して紫外線のみ照射したものは 72 時間後に蒲鉾の側面に細菌が僅かに発生し，96時間後にカビが僅か発生した程度であつた．防腐

剤と紫外線とを併用したものの中では，1％の過酸化水素水を塗布したものが最も有効で，本試験期間中には何等異常を認めなかつた．次いで BHC，POB，H_2O_2+MA，GF を塗布したものが有効で，96時間後に蒲鉾の側面に細菌が僅か発生した程度であつた．V.K_3 を塗布したものの効果は紫外線のみを照射したものの効果と全く差がなかつた．

4．赤外線，紫外線，防腐剤及びセロファン包装の併用効果

A．無包装の場合に於ける効果

Table 6 及び 7 に示した赤外線の効果は殺菌だけでなく，恐らくは照射表面の水分を減少して細菌の発育を遅延させる効果も大きいものと推察される．また赤外線を照射するとカビの繁殖を有効に抑制することが注目された．これに対し 2,500～2,600Å の紫外線の効果は細胞核の変性に原因するのではないかと推察されている．しかし Table 8 の結果をみると紫外線は細菌の発育を有効に抑制するが，カビの発育に対しては余り効果がみられなかつた．防腐剤の作用機構は明らかでないが，それぞれ特有の抗菌スペクトラムをもつことは周知の通りである．かように作用機構の異る両輻射及び防腐剤を併用すれば，防腐効果を一層増大させるのではないかと期待される．

依て本試験では試験開始日に防腐剤を蒲鉾の表面に塗布した後，先ず赤外線を，次いで紫外線を蒲鉾の上面にそれぞれ 10 分間照射した．なお紫外線のみは試験中毎日照射を続けた．防腐試験の結果は Table 10 に示すように，対照区は 24 時間後に異臭を発し，48 時間後には細菌が多発し，120時間後の揮発性塩基は 67.7 mg％ であつた．これに対し赤外線及び紫外線を照射したものは72時間後にカビが僅かに発生したが，その後カビは発育せず，細菌は本試験期間中には認められなかつた．また120時間後の揮発性塩基は 14.5 mg％で，赤外線及び紫外線の照射は顕著な鮮度保持効果を示した．防腐剤，赤外線及び紫外線を併用したものはいずれも，赤外線及び紫外線のみを照射し防腐剤を塗布しなかつたも

Table 10. Effect of combination of infrared ray, ultraviolet ray and preservatives.

Time on incubation at 22～29°C (hr)		24			48				72				96			120
Quality / Treatment		"Neto"	Fungi	Odor	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %	"Neto"	Fungi	Odor	V.B.N mg %
Ethanol*		—	—	±	++	—	+	28.8	++	—	+	34.8	++	++	+	67.7
Irradiation by infrared and ultraviolet ray	None	—	—	—	—	—	—	6.7	—	±	—	7.7	—	±	—	14.5
	BHC*	—	—	—	—	—	—	7.3	—	—	—	9.4	—	±	—	17.8
	IAG*	—	—	—	—	—	—	7.3	—	—	—	11.5	—	±	—	18.1
	POB*	—	—	—	—	—	—	8.3	—	—	—	8.4	—	±	—	16.8
	H_2O_2*	—	—	—	—	—	—	5.8	—	—	—	9.8	—	±	—	19.6

* See Table 1. The upper part of "Kamaboko" was subjected to a 10-minute irradiation with a 500-W infrared ray and a 15-W ultraviolet ray lamp at a 30cm distance. The ultraviolet ray irradiation was carried out daily.

のに比すと，カビの発生は遅かつたが，発生後のカビの発育は変らず，また120時間後の揮発塩基は僅かに多かつた．従つて防腐剤の効果は殆んどないものと認められる．

B．セロファン包装した場合の効果

本試験は2回行つた．試験Ⅰでは防腐剤を蒲鉾の表面に塗布した後，直ちにセロファンで完全に包装し，赤外線及び紫外線を蒲鉾の上面に10分間照射し，24～30°C の室に放置した．なお紫外線は試験期間中毎日照射した．官能検査及び揮発性塩基の値は Table 11 に示すように，無処理の対照区は24時間後に細菌が発生し，48時間後に多発した．またエタノールを塗布して包装した対照区は48時間後に細菌が多発し，120時間後の揮発性塩基は 50.0 mg% であつた．包装して赤外線及び紫外線を照射したものは72時間後に蒲鉾の側面に細菌が発生したが，その後の細菌の発育は緩慢であつた．120 時間後の揮発性塩基は

Table 11. Effect of combination of infrared ray, ultraviolet ray, preservatives and casing.

Experiments	Treatment	Casing ○ With cover × Without	Time on incubation at 24～30°C (hr) 24 "Neto"	24 Fungi	48 "Neto"	48 Fungi	48 V.B.N mg %	72 "Neto"	72 Fungi	72 V.B.N mg %	96 "Neto"	96 Fungi	96 V.B.N mg %	120 "Neto"	120 Fungi	120 V.B.N mg %
I†	None	×	±	—	+	±	10.6	##	##	17.8			20.6	##	##	29.8
	Ethanol*	○	—	—	##	—	22.4	##	±	14.4			31.4	##	##	50.0
	Irradiated upper part only: None	○	—	—	—	—		±	—	7.7			6.7	##	+	10.5
	Irradiated upper part only: BHC*	○	—	—	±	—		±	—				12.5	+	+	12.0
	Irradiated upper part only: IAG*	○	—	—	±	—		±	—				12.3	+	+	14.3
	Irradiated upper part only: POB*	○	—	—	±	—		±	—				16.6	+	+	12.6
	Irradiated upper part only: NP*	○	—	—	±	—		±	—				12.3	##	+	12.5
	Irradiated upper part only: H_2O_2*	○	—	—	—	—		±	—				10.1	+	+	11.3
II§	None	×	—	—	—	+		—	##		+	##				
	None	○	—	—	—	±		+	+		##	##				
	Irradiated all over the surface: None	○	—	—	—	—		±	—		+	±				
	Irradiated all over the surface: POB*	○	—	—	—	—		—	—		—	—				
	Irradiated all over the surface: H_2O_2*	○	—	—	—	—		—	—		±	—				
	Irradiated all over the surface: H_2O_2 +MA*	○	—	—	—	—		—	—		—	—				
	Irradiated all over the surface: GF	○	—	—	—	—		—	—		±	±				
	Irradiated all over the surface: VK_3	○	—	—	—	—		—	—		±	±				

* See Table 1 and 5. † "Kamaboko" was covered with cellophane just after the application of chemicals, and then subjected to a 10-minute irradiation with a 500-W infrared ray and a 15-W ultraviolet ray lamp at a 30cm distance. The surface of "Kamaboko" was smeared with the chemicals after a 15-minute irradiation with a 500-W infrared ray lamp, and covered with cellophane and then subjected to a 15-minute irradiation with a 15-W ultraviolet ray lamp at a 30cm distance. The ultraviolet ray irradiation was carried out daily.

10.5 mg% で，顕著な鮮度保持効果を示した．防腐剤，包装，赤外線及び紫外線を併用したものでは，過酸化水素水を塗布したものが包装，赤外線及び紫外線を併用したものと同程度の効果を示したが，その他の防腐剤を塗布したものは48時間後に蒲鉾の側面に細菌が発生し，包装，赤外線，紫外線併用区より多少効果が劣るようである．エタノール塗布後直ちに包装した対照区が，無処理の対照区より鮮度が著るしく低下したことを思うと，これらの効果の減弱は薬品塗布からの水分増加及び包装による水分保持が細菌の発育を促進したためと思われる．また試験区は全て先ず蒲鉾の側面に微生物が発生した．依て試験Ⅱでは赤外線を蒲鉾の全面に15分間照射した後，防腐剤を塗布し，数分後セロファンで包装し，紫外線を全面に15分間毎日照射した．試験の結果は Table 11 に示すように，無処理及び包装した対照区は48時間後にカビが発生した．これに対し包装，赤外線及び紫外線を併用したものは72時間後に蒲鉾の側面に細菌が僅か発生し，96時間後にカビが発生し初めた程度であり，鮮度保持効果が明らかに認められた．防腐剤，包装，赤外線及び紫外線併用区はいずれも顕著な効果を示した．POB 或いは H_2O_2+MA を塗布したものは最も有効で，本試験期間中は微生物の発生が全く認められなかつた．次いで H_2O_2, GF, V.K_3 の順に有効であり，96時間後に微生物の発生が認められたにすぎない．

IV. 総　　括

1．無包装の板付無糖蒲鉾の表面に数種防腐剤を塗布したところ，過酸化水素水，過酸化物価の高いオレイン酸及び第四級アミンによる鮮度保持日数は，対照の1日に対し2～3日で，多少鮮度保持効果を示した．

2．セロファン包装によつて二次的汚染は防止されることが認められた．しかし包装と防腐剤を併用した場合の内，防腐剤塗布後直ちに包装したものはすべて無包装の対照より変敗が早かつた．他方予め加温した蒲鉾に防腐剤を塗布し，数分後包装したものでは，過酸化水素水，パラオキシ安息香酸ブチルエステル及びグアノフラシンが僅かに有効であつた．

3．無包装蒲鉾の上面に赤外線を照射したものの鮮度保持日数は，無処理の対照の1日に対し2日であつた．なお微生物の発生個所は蒲鉾の側面に限られていた．赤外線と併用した防腐剤の中では，1%過酸化水素水が有効で鮮度保持日数は3日であつた．

4．セロファン包装した蒲鉾の上面に赤外線を1回照射した場合は，赤外線照射の効果は全く認められなかつた．またこの包装の場合には防腐剤の効果も認められなかつた．しかし赤外線を毎日照射した場合は有効に微生物の繁殖を抑制した．

5．無包装の蒲鉾の上面に紫外線を毎日照射したものの鮮度保持日数は，無処理の対照の1日に対し4日で顕著な鮮度保持効果を示した．なお防腐剤を併用した場合，防腐剤による効果は全く認められなかつた．

6．セロファン包装した蒲鉾の上面に紫外線を毎日照射したところ，微生物の繁殖を抑制し鮮度保持効果を示した．防腐剤の塗布後，直ちに包装し紫外線を照射したものの中では，1%過酸化水素水を塗布したものが最も有効で，鮮度保持日数は無処理の対照の1日，包装した対照の2日に対し3日であつた．他の防腐剤の効果は認められなかつた．蒲鉾の

上面に紫外線を照射した場合，微生物は常に先ず蒲鉾の側面に発生した．

7．赤外灯で加温した蒲鉾に防腐剤を塗布し，数分後包装して紫外線を毎日蒲鉾の全面に照射したところ，1％過酸化水素水を塗布したものが最も有効で，無処理及び包装した対照区が2日後に変敗したのに対し全試験期間の4日間異常が認められなかつた．紫外線のみを毎日蒲鉾の全面に照射したものは3日間鮮度を保持した．

8．無包装の蒲鉾の上面に赤外線を照射した後，紫外線を蒲鉾の上面に毎日照射したものは，無処理の対照区の1日に対し4日間鮮度が保持された．しかし防腐剤を併用した場合，防腐剤の効果は認められなかつた．

9．セロファン包装した蒲鉾の上面に赤外線を照射した後，紫外線を毎日照射したものは，無処理の対照が1日，包装した対照が2日鮮度を保持したのに対し，3日間変敗せず有効であつた．防腐剤塗布後直ちに包装し，赤外線及び紫外線を併用した場合は防腐剤の効果は全く認められなかつた．

10．赤外線を蒲鉾の全面に照射した後防腐剤を塗布し，数分後包装して紫外線を蒲鉾の全面に毎日照射した場合は，パラオキシ安息香酸ブチルエステル，過酸化水素水＋マレイン酸は最も有効で，無処理及び包装した対照区の鮮度保持日数2日に対し，本試験期間の4日間全く変敗しなかつた．

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Summary

"Kamaboko" is one of very popular foods in Japan. It is made by cooking the fish muscle pulp well mixed with salt, starch and tasteful ingredients, such as sodium glutamate and sugar or saccharine. It is a serious problem from the hygienic as well as producer's stand point that the kamaboko is readily spoiled and usually possesses only one- or two-day storage life at room temperature. The present study deals with the application of preservatives, casing and various radiations in order to increase the keeping quality of this food. The results obtained can be summarized as follows:

1. Among preservatives which were applied on the surface, hydrogen peroxide, oleic acid having high peroxide value and quaternary ammonium compounds were effective in prolonging the storage life to two to three days in contrast to one day in the control. (See Tables 1～4).

2. The casing with cellophane was found effective to elimination of the secondary contamination. The combination of the casing and smearing of preservatives on the surface was effective only when hot surface of the kamaboko was treated with solutions of preservatives followed by casing after few minutes' standing, while a group in which the casing was made just after the treatment with preservatives was rather inferior to the control group without casing. (See Table 5).

3. When the surface was irradiated with infrared ray, the storage life was prolonged to two d ys in contrast to one day in the control. It was observed that micro-organisms were not developed on the upper surface but only on the side. The application of hydrogen peroxide followed by the irradiation was slightly more effective than irradiation only. (See Table 6).

4. Irradiation by infrared ray on the surface of a cellophane-covered kamaboko gave no improvement as compared with cellophane covered control without irradiation. No improved results in this case was observed due to the application of preservatives before casing with cellophane. Daily irradiation, however, resulted in a marked prolongation of the storage life. (See Table 7).

5. Daily irradiation by ultraviolet ray on the surface of the kamaboko without casing was effective to prolonging the storage life as long as four days in contrast to one day in the control group without the irradiation. No effect of keeping quality was obtained due to the application of preservatives. (See Table 8).

6. Daily irradiation on the surface of the cellophane-covered kamaboko by ultraviolet ray was effective to prolongation of the storage life to two days as contrasted to one day without irradiation. Among preservatives applied on the surface before casing and irradiation, hydrogen peroxide was found the most effective. (See Table 9).

7. Daily irradiation by ultraviolet ray all over the surface of the kamaboko which was covered with cellophane after few minutes following the smearing of preservatives on hot surface was effective to prolongation of the storage life to three days in contrast to two days in the control without irradiation of ultraviolet ray. Among the preservatives applied, hydrogen peroxide and butyl p-oxybenzoate were more effective than any other one under test. (See Table 9).

8. Daily irradiation on the surface of the kamaboko without casing by infrared ray followed by ultraviolet ray was able to make the storage life four days as contrasted to one day in the control group without irradiation. The combination of this procedure with preservatives gave no improvement. (See Table 10).

9. Daily irradiation of infrared and ultraviolet ray on the surface of the cellophane-covered kamaboko was able to prolong the storage life to three days in contrast to two days in the covered control without irradiation. When the casing was made just after the application of the preservatives, no better effect was resulted. (See Table 11).

10. However, when infrared ray was irradiated before the application of the preservatives, somewhat effective result was obtained; particularly the application of butyl p-oxybenzoate and hydrogen peroxide mixed with maleic acid were found to keep the quality for four days. (See Table 11).

工場廃水の淨化方法に関する研究—III. パルプ廃液の酸添加処理限度並びに放流についての具体的考察

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Studies on Purification of Industrial Waste—III. A critical study on amount of waste to be treated and of effluent permissible to be discharged to the river.

Katsumi KAWABE and Tetuo TOMIYAMA

In order to estimate appropriate volume of the waste to be treated, determination has been made of the volume and concentration of the original waste liquor from the digester of 1.5 tons capacity for knot or chips and of the washing from diffuser.

Based on a minimum concentration that brings about a dark coloration in *Carassius auratus*, it has roughly been estimated that 1.4 tons out of 3.1 tons of the original waste should be subject to the acid treatment, provided that about 1.3 tons of the original waste be used for conglomerating briquet. Approximate amount of hydrochloric acid needed for the treatment was calculated to be 24 liters of 38 per cent hydrochloric acid when 1.4 tons of waste liquor from one batch of digestion be reused for cooking the used paper and straw.

It has been shown that precipitated poisonous ingredients of the waste could not be extracted with hot water and that the suspension of the precipitate gave as much harmfulness as the original waste. It follows, accordingly, that when the waste be discharged without filtration after the acid-treatment, the precipitate deposited over the drainage-basin would give some harmful effect on aquatic organisms.

第一報と第二報とに於いて，ノット或いはチップのアルカリ蒸解廃液を酸添加処理で淨化する最適条件並びに廃液及び洗滌残渣の利用法などに関する研究結果を報告した。本報では先ず前記S工場の実際の廃液量，濃度及び流入河川の水量について具体的検討を加え，廃液の毒性を考慮した安全放出量の算出を試みた。また，工場内外での廃液の利用並びに工場の具体的工程，及び廃液の量並びに濃度を考慮した場合の実際的処理量を検討し，また最適処理過程について考察を行ったのでその結果を報告する。

I. パルプ廃液の量並びに濃度についての具体的考察

Digester で蒸解されたパルプは廃液と共に Diffuser に移されて洗滌されるが，廃液処理に当っても一応原廃液（洗滌水を含まぬもの）と洗滌廃液とを分けて考えた方が合理的である。そこで，原料仕込量 1.5 トンの Digester で蒸解完了後 Diffuser に移し，自然流出するパルプ中の大部分の廃液を除いた後，Diffuser を洗滌水で満たし，これを同底部から，2吋パイプを通じて排出した時の洗滌廃液の濃度及び排出量の時間的変化を追跡した（Table 1.）。次に Table 1 のヨード消費量を濃度の基準とし，排出開始後の各時間までの累算排出量及び累算濃度変化（Fig. 1.），及び洗滌廃液中の原廃液の累算量（Fig. 2.）を算出した。

1954年11月1日受理

Table 1. Rate of discharge of waste from diffuser and of decrease in density, color and oxidizable matter.

No. of sample (n)	Sampling time-period (min.)	Temperature (°C)	Time required for filling 70 l. barrel (min.)	Rate of discharge (l./min.)	Specific gravity (Baume)	Color intensity of 100 times diluted sample ($-\log T$ at 420 mμ)	Iodine consumed by sample (cc. 0.1N I/cc.)	Conc. of waste as estimated by color intensity (%)	Conc. of waste as estimated by Iodine consumed (%)
0		80-64			7.0	0.620	13.3	100	100
1	0	75	22	190	6.5	0.600	12.5	96.7	94.0
2	5	70	38	110	5.7	0.557	11.5	90.0	86.5
3	10	60	44	95	4.5	0.400	9.1	64.6	68.4
4	15	55	50	84	3.3	0.295	6.9	47.5	52.0
5	20	47	60	70	2.8	0.226	5.5	36.5	41.5
6	25	34	82	51	2.2	0.140	3.5	22.6	26.3
7	32	25	105	40	1.4	0.102	1.8	16.6	16.1
8	40	19	150	28	1.0	0.070	1.3	11.6	9.8
9	50	17	182	24	0.8	0.057	0.3	9.2	2.4

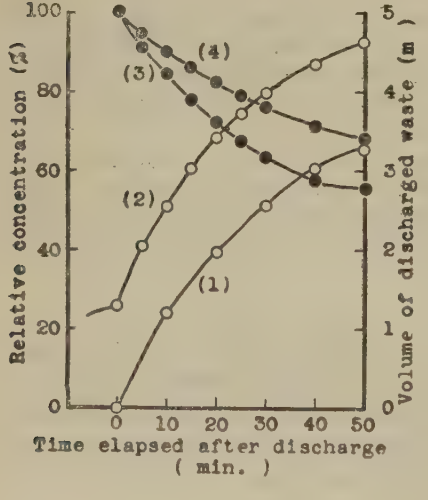

Fig. 1. Cumulative volume and concentration of washing-discharge.
(1) Cumulative vol. of washing.
(2) Sum of vol. of original waste and cumulative vol. of washing.
(3) Decrease in cumulative conc. of mixture of original waste and washing.

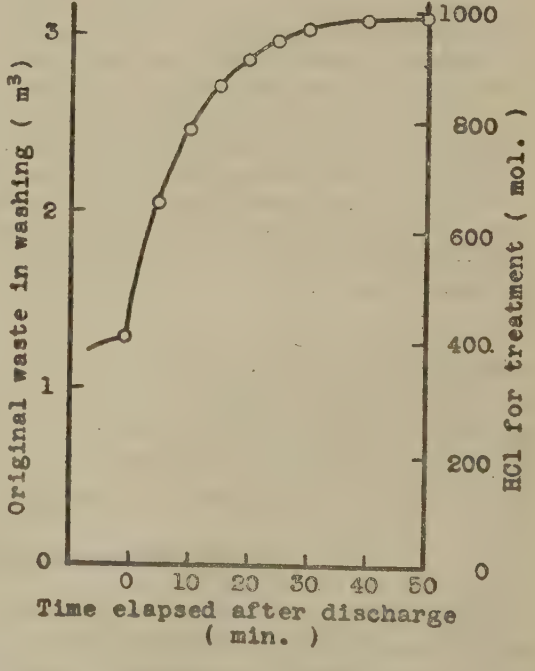

Fig. 2. Increase in cumulative volume of original waste in washing.

Fig. 1. 及び Fig. 2. に於いて, (1) : $\sum_{1}^{n} V_n$ (洗滌廃液のみの各時間までの累算排出量), (2) : $\sum_{0}^{n} V_n$ (原廃液と洗滌液の各時間までの累算排出量の和), (3) : $\sum_{1}^{n} C_n V_n / \sum_{1}^{n} V_n$ (各時間までの排出洗滌廃液の累

算濃度), (4): $\sum_0^n C_n V_n / \sum_0^n V_n$ (原廃液と各時間までの洗滌廃液とを混合した時の累算濃度), (5): $\sum_0^n C_n \cdot V_n/100$ (各時間までの廃液を原廃液濃度に換算した時の累算排出量)。こゝに, C は各試料に含れる原廃液の濃度 %, V は排出量 l. を示し, n は Table 1. に於ける試料番号を示す。また Fig. 2. に於いて単位廃液量に対する所要塩酸量のスケールをあて嵌めれば塩酸の所要量が判るが, この図に示したのは Knot-pulp waste を対象とした塩酸量である。

II. パルプ廃液が流入する河川の水量と廃液の安全放出量に関する考察

実際問題としてこの廃液の全量を酸添加処理することは困難である。そこで廃液の毒性と受水区域の水量から安全放出量を算出してみた。

1. 河川の水位から流水量を算出する試み： 廃液の放出量は受水区域の流水量の変化に応じて調節すべきであるから, まず流水量を測定する必要がある。しかし, 常に流水断面と流速とを測定して流量を算出することは繁雑である。そこで, 水深のみの測定値から流水量を算出する近似式を導いた。

いまこの工場横の川の場合についてみると, 現場試験時に実測した断面図は Fig. 3. に示す通りで, 同時に測定した st. C に於ける表面流速は 0.44 m/sec. であつた。従つて測定時の流水量 Q は $Q=A\times V_m$ (A は断面積, V_m は平均流速でほゞ表面流速の 0.8 倍と考えてよい) から, 約 2.07 m³/sec. となる。

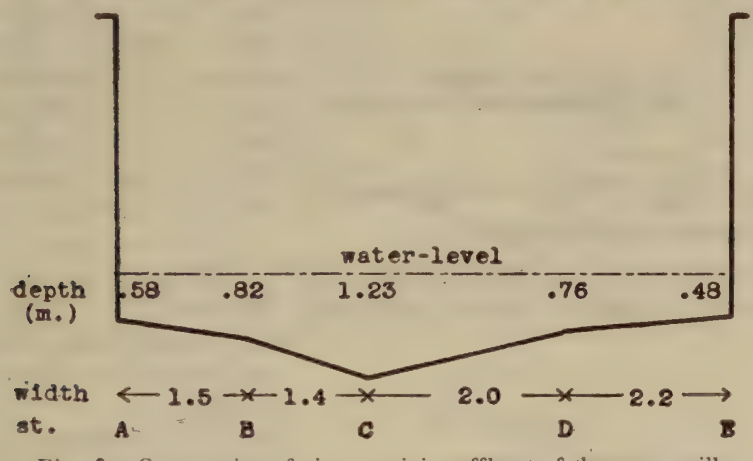

Fig. 3. Cross section of river receiving effluent of the paper mill.

次に, 流速は一般に勾配と護岸の性質との函数と考えてよく, これらの間に (1) 式が成立する[3]

$$V=C\sqrt{RI} \qquad (1)$$

こゝに, V : 流速, C : 比例恒数, R : 径深 ($=A/S$) 但し S は潤辺 (横断面に於ける護岸と流水との接触部分の長さ), I は勾配に関する函数である。

然るに, I は特殊の場合を除き同一場所では近似的に一定と考えられ, また C については Manning らの実験式があるが, この場合には実測値から求められるので, いま $C\sqrt{I}=C'$ とおけば (2) 式が成立する。

$$V=C'\sqrt{R} \qquad (2)$$

この式に実測値を代入して C' を求めれば $C'=0.407$ となり, (2) 式は次の (3) 式で表わされる。

$$V=0.407\sqrt{A/S} \qquad (3)$$

こゝに, Fig. 3. の C 地点に於ける深度を流量測定の基準にとることとし, その深度を h 米とすれば, $h>0.75$ に於いては Fig. 3. から S 及び A を次の如く算出できる。

$$S=8.1+2(h-1.23)=2h+5.74, \quad A=5.91+7.04(h-1.23)=7.04h-2.70$$

$$\therefore \quad V=0.407\sqrt{(7.04h-2.70)/(2h+5.74)}$$

$$\therefore \quad Q=(2.87h-1.13)\sqrt{(7.04h-2.70)/(2h+5.74)} \quad \text{m}^3/\text{sec.} \qquad (4)$$

即ち, (4) を用うれば, 水深のみの測定によつて流量の算出が容易にできることになる。

2. 廃液の毒性と安全放出量の検討： 次に Knot pulp waste について, ヒブナ仔魚に対する二十四時間致死量をみたところ, 追つて報告するように約 800 倍稀釈濃度であつた。しかし, その毒物は神経系統に作

用し，2,000 倍稀釈程度までは体色が黒変し，明らかに嫌忌の状態がみられたので，仮りに安全稀釈濃度を 3,000 倍とする。

いま渇水期の安全放出量を計算してみると，渇水期には前記の川で水深は Fig. 3. の C の地点で約 0.8 米，即ち一日当り 63,940 m³ となり，この値を前記稀釈倍率で割れば約 21 m³ となる。この値は同工場の全力操業時に於ける廃液量（原廃液濃度で）約 25 m³/day に近いので，完全な均一混合を計り，また等量宛放流すれば全部放流してもよいかのようである。しかしこの値は一次的毒物のみを考慮した場合であつて，なお，長期に亘つて検討すべき重要なものとして，B.O.D. の上昇，流出短繊維の附着や沈澱などの外に，上記の一次的毒物が下流域で沈降堆積して二次的な毒性の原因になることを考慮する必要がある。このことは第一報に示したように，中性附近でこの毒物が沈澱することから考え特に注意を払うべきである。

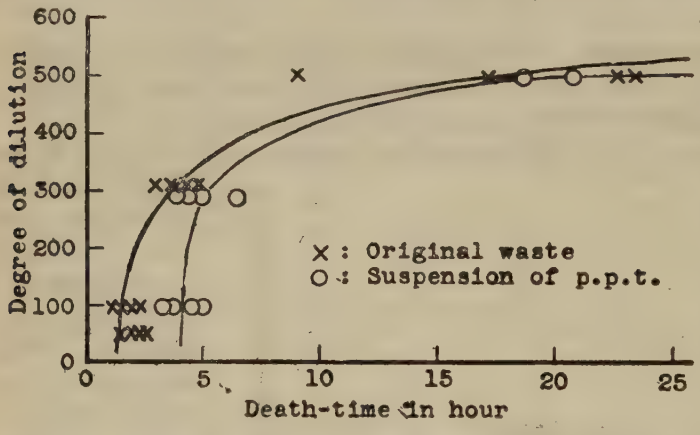

Fig. 4. The effect on *Carassius auratus* GÜNTHER "Hibuna" of diluted waste and suspension of precipitate formed on acidification.

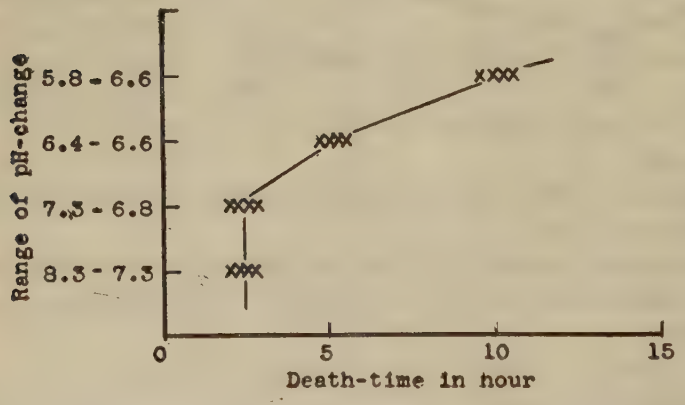

Fig. 5. Change in poisonous effect on *Carassius auratus* GÜNTHER "Hibuna" of suspension of precipitate obtained by adding HCl.

また沈澱の生成や堆積によつてどの程度の毒物が現われるかを知るために，酸性で析出する沈澱物の懸濁液がヒブナ仔魚に及ぼす毒性について試験し，Fig. 4. 及び Fig. 5. に示す結果をえた。沈澱物を水で煮沸抽出後に濾したものは毒性を示さなかつたが，水中に懸濁させた場合にはアルカリ性では勿論毒性を示すが，酸性でも原廃液とほとんど変らぬ毒性を示した。

これらのことから最初述べたような安全放出量という考え方は非常に危険であることが判るが，上述の条件を考慮した安全放出量を算定することもまた極めて困難である。そこで一応 Fig. 2. に示した総排出量の内，最初の洗滌水を含まぬ部分約 1.3 m³（一釜当り）を第二報に述べたように煉炭の粘結剤として利用し，残りの 1.8 m³ を pH 4.5 迄酸添加して処理するとすれば，Knot-pulp waste の場合，塩酸で滴定した結果 pH 8 迄中和するに必要な塩酸量は 257 Mol./m³ であり，pH 8 から 4.5 までに必要な塩酸量は 78 Mol/m³ であるから，いま N m³ を酸処理し，その濾液を残りの稀薄廃液と混じて互いに中和させ，中和後の沈澱を濾過又は沈降させて放流するとすれば，N は次式から求められる。

$$78N-257(1.8-N)=0$$

これより $N \fallingdotseq 1.38$ となる。つまり 3.1 m³ の内で実際には約 1.4 m³ を酸処理の直接の対象とすればよいことになる。また，この 1.38 m³ を洗滌廃液の中の濃厚部からとるとすれば，実際の濃度での廃液量は約 1.8 m³ であり，その濃度は原廃液の約 77 %，遊離アルカリは約 $0.2N$ となる。そこで，これを第二報

で述べたように，故紙蒸解の仕込液として利用した後，その廃液を同様酸処理するとすれば，この工程で遊離アルカリは約 40 % に減少するから，処理塩酸量は $(257\times0.4+78)\times1.38\times250$ Mol. となる。これを濃塩酸 (38%) に換算すれば，約 24 *l*. を必要とすることゝなる。

III. パルプ廃液の具体的処理系統

第二報の結果並びに本報の結果を綜合して，[illegible]の廃液の実際的処理方法として Fig. 6. に示すような系統図が得られる。即ち，Diffuser から排出する廃液のうち洗滌水の混じていない部分は煉炭の結結剤などに利用し，洗滌廃液を二つに分け，初め出てくるボーメ 7～4.5 の濃厚部は故紙蒸解の仕込液として使用した後，直ちに処理槽に移して酸添加処理する。その時できる上澄液をとり，残りは更に濾過槽に移し，濾液は前記上澄液と共に沈澱池に送り，Diffuser から直接こゝに排出したボーメ 4.5 以下の廃液と混じて中和させ，その上澄液を放流する。なお漂白液製造槽の漂白液残渣は，処理濾液を沈澱池に送る途中で混ずる。

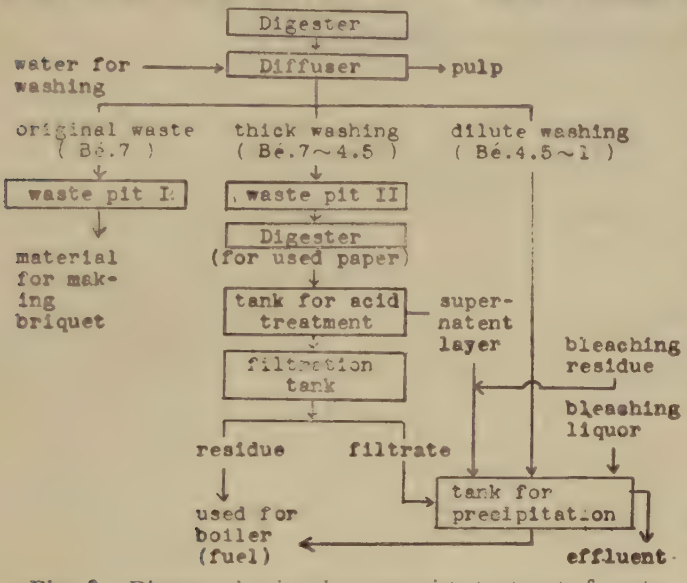

Fig. 6. Diagram showing the appropriate treatment of waste from the paper mill

要　　約

1. パルプ廃液を原廃液と洗滌廃液とに分け，それぞれについて具体的利用及び処理量の検討を行つた。
2. 原廃液濃度換算で 3.1 m³ の廃液中 1.38 m³ を処理すればよいことが判つた。但し，故紙の蒸解に廃液の一部を使用した後処理すれば，酸の処要量は約 50 % 節減できる。
3. 廃液を放流する工場槽の川について，水深の測定のみにより流水量を算出する近似式を導いた。
4. 廃液中の毒物は中性以下で沈澱し，その毒物は熱水に溶けないが，沈澱の懸濁液は原廃液の毒性とほとんど変らなかつた。この毒性は pH の上昇と共に増すが，酸性に於いても懸濁液の毒性は残つた。このことから考えて，受水区域の流水量並びに一次的毒性から判定した安全放出量を放流したとしても，酸処理後濾過しなければ B.O.D. の増加以外にこの毒物についての二次的危険性が残る惧れがある。
5. 工場の実際的工程に於ける廃液の利用並びに処理過程を検討した。

本研究の遂行に協力を惜しまれなかつた齊藤武業 K.K. に謝意を表し，また研究費の援助を与えられた福岡県当局に深謝する。

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工場廃水の浄化方法に関する研究 —Ⅳ.
製紙工場の漂白廃液について

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工場廃水の淨化方法に関する研究—Ⅳ.
製紙工場の漂白廃液について

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Studies on Purification of Industrial Wastes—Ⅳ.
Treatment of wasted bleaching liquor in paper mill

Katsumi KAWABE and Tetuo TOMIYAMA

It has well been known that the harmful effect of paper mill waste on aquatic organisms is mainly due to its active chlorine in the effluent. Estimations, accordingly, have been made of decrease in the active chlorine in the effluent from the beater, in sediment of bleaching liquor tank, and also in effluent from the factory.

It has been found that 90 per cent of the active chlorine added into the beater was consumed within one hour, 5-20 ppm being present in the effluent from the beater. Since this amount was further decreased to below 1 ppm after mixing with alkaline pulp-waste liquor, it can be concluded that pollution by the residual chlorine from the beater will not be of a serious nature. However, the sediment in the bleaching liquor tank has usually been discharged without treatment which resulted in a sudden increase in active chlorine in the effluent from the factory. The present data show that the active chlorine in the sediment can be removed by mixing it with the acid-treated pulp-waste liquor.

製紙工場廃水の内で最も毒性が強いと考えられるのはパルプ廃液であるが，この他に製紙工程に於ける廃水がある。この廃水の毒性は，主として遊離塩素の存在によるものと思われる。この種の廃水には Beater からの漂白並びに洗滌廃水の外に，漂白液製造槽の掃除の際に排出される濃厚な廃水がある。前者の遊離塩素濃度は低いと思われるが，常にほゞ恒量ずつ多量に排出されている。しかし後者は一時に排出されるもので，遊離塩素濃度は高い筈である。後者については濃縮焙焼後石灰として回収することも考えられるが，本報では還元性物質との混合により遊離塩素の活性を失わせてその毒性を減ずる方法について検討した。

実験結果及び考察

1. Beater からの廃水

Beater に移されたパルプは約2トンの水に懸濁され，漂白液を加えたのち楕円回路を回転叩解されながら漂白される。漂白液は予め漂白液槽に貯えられ，仕込み時に一定量を加え約一時間漂白を続けた後，一定量の洗滌水をそゝぎながら更に四，五時間叩解洗滌する。この際洗滌水の注入によって溢れた水が洗滌廃水として常時流出している。この間漂白並びに洗滌の進行と共に廃水の濃度も常に変化しているので，一時間毎に Beater 内の液を採取し，その中の遊離塩素をヨード法により定量した。

Table 1. Change of free chlorine during bleaching liquor in the beater.

Sample	pH	Free chlorine	
		cc. 0.1 N I/cc.	Cl-ppm.
Bleaching liquor	11.4	680.00	24,150
Start of bleaching		20.50	726
After 1 hour	8.4	2.25	80
After 2 hours	5.8	0.47	12
After 3 hours	5.8	0.23	8
After 4 hours	5.8	0.23	8
Sediment of bleaching liquor	11.4	742.00	26,300

1954 年 11 月 1 日受理

その結果は Table 1. に示すように残留する遊離塩素の量は漂白開始から一時間の間に最初の約 90 %が消費され，以後洗滌水の注入と共に遊離塩素濃度も次第に小さくなり，洗滌を開始してから約一時間後にはほゞ恒量になる。この時の遊離塩素濃度は約 10 ppm. 程度である。廃水として出るのは洗滌水を注入し始めてからであつて，次第に濃度を減じた廃水が一定量ずつ排出されるが，実際には Beater は4基あつて各各別個に操業しているので，各 Beater からの廃水が集まつた排水溝ではこれ等が平均されており，遊離塩素の実測値は5乃至 20 ppm. であつた。またこの廃水が現在の沈澱池を通つて川に放出される場所ではほとんど 1 ppm. 以下であつた。これは沈澱池で Digester その他からの有機質の廃液によつて還元されるためであると考えられる。

高安氏[1]によれば，鯉に対する遊離塩素の致死量は 0.2 ppm. であり，以上の各測定値から考えてこの工場の Beater 廃水に含まれる遊離塩素が魚類の斃死原因になるとは考え難い。

2. 漂白液残渣

この工場では直径約2米，高さ約 1.5 米のコンクリート槽に漂白粉の懸濁液を入れ，これに塩素ガスを吹込で活性漂白液を作つている。この際不溶性残渣が槽底に溜り，2日間に約1トンに及ぶため2日に1回位これを排除する。これは相当多量な遊離塩素を含んだ廃液をなしている。現在この廃液は沈澱池で他の廃水と混合した後，川に放流している。

この残渣は漂白液に懸濁した状態で排出されるので，この懸濁液を試料としヨード法により遊離塩素の定量を行つたところ、26300 ppm. -Cl であつた。従つてこれを一時に放流すれば魚類斃死の原因となる惧れがある。実際現在までに下流域で起つた漂白廃液による魚類斃死の原因は，この残渣を一時に放出したためではないかと推察される。

この廃液をパルプ廃液と混合すれば，パルプ廃液は大きな還元力を持つているので，遊離塩素を除去しうるであろうとの観点から，両者の酸化並びに還元力の当量値を求めてみた。即ち，小型試験管に Knot-pulp waste, chips-pulp waste 並びにそれ等の酸処理後の濾液をそれぞれ 1cc. 宛1個の試料について4本とり，これに夫々前記漂白廃液 2 cc. あるいは 1 cc. を添加してよく振盪した後室温に放置し，1, 3, 6, 及び 22 各時間後の残存遊離塩素量を測定し Table 2. に示すような結果を得た。

Table 2. Rate of decrease in free chlorine (cc. 0. 1 N I/cc.) on mixing bleaching liquor with each pulp wastes.

Sample	Volume of sample	Bleaching liq. added	Free chlorine				
			at start	Remained after			
				1 hour	3 hours	6 hours	22 hours
Knot-pulp waste	1 cc.	2 cc.	13.60	10.70	7.40	4.95	2.66
〃 treated	1	1	6.80	3.10	1.30	0.52	0.04
Chips-pulp waste	1	2	16.60	6.00	5.40	4.55	2.83
〃 treated	1	1	6.80	2.30	1.10	0.46	0.08

次にパルプ廃液並びにその酸添加処理濾液のヨード消費量に対して，漂白液残渣懸濁液にヨードカリを加えて遊離するヨード量を比較対照すると Table 3. に示すようになる。即ち漂白残渣の遊離塩素は，その 1.5 倍の酸処理濾液により除去される計算となる。また，Knot-pulp waste とその酸添加処理濾液とについて過マンガン酸カリ消費量とヨード消費量とを比較すると，Table 4. に示すようにヨード消費量の方が一般に小さい。しかし遊離塩素の酸化力は過マンガン酸カリよりは小さく，ヨードよりは大きい筈であるから，各種廃水の還元力と当量の酸化力を示す漂白液残渣の量はほゞ Table 5. に示した範囲と考えてよいであろう。

Table 3. Oxidation and reduction-capacity of bleaching liquor and pulp-waste expressed as cc. 0.1 N I/cc. sample.

Sample	Oxido-reduction capacity cc. 0.1 N I/cc.	Calc. amt. of each pulpwaste needed to remove free chlorine in bleaching liquor
Free Cl in sediment of bleaching liquor	7.42	
Knot-pulp waste	16.20	0.46 cc./cc.
〃 treated	4.00	1.85
Chips-pulp waste	32.00	0.23
〃 treated	5.00	1.48

Table 4. Comparison of $KMnO_4$ consumed with iodine consumed of pulp-waste and treated waste.

Sample	Oxygen consumed from $KMnO_4$ (cc. 0.1N $KMnO_4$/cc.)	Iodine cons. (cc. 0.1N Ioidne/cc.)	$KMnO_4$ cons. / Iodine cons.
Knot-pulp waste	28.30	5.40	5.24
Ditto treated	3.40	1.17	2.91

Table 5. Volume of bleaching liquor needed to oxidize each wastes.

Wastes and treated waste	Volume of bleaching liguor needed to oxidize each wastes
Knot-pulp waste	2.2～11.4
〃 treated	0.5～11.6
Chips-pulp waste	4.5～22.7
〃 treated	0.7～ 2.0

パルプ廃液と漂白液残渣との反応速度は温度及び pH によつて非常に異ると考えられる。温度については van't Hoff の反応速度-温度則に従うと思われるので，本報に於ては特に pH の影響を検討した。即ち，Knot-pulp waste の 10 倍稀釈液 10 cc. 宛を試験管にとり，漂白原液各 1 cc. 宛を注加した後更に稀塩酸(約 3.5 %) を加えて pH を 11.0(無添加)，6.6 及び 4.0 に夫々調製し，よく振盪後 3 時間室温に放置した時の遊離塩素の減量をヨード法により定量し，Table 6. に示すような結果を得た。即ち，pH 6.6 及び 4.0 に於ては pH 11.0 の場合に比べてそれぞれ約 1.4 及び 1.6 倍の消費量を示しており，またパルプ廃液はその処理濾液に比べ酸化され難い事，また廃液及び処理濾液の pH はそれぞれ 13 附近及び 4.5～5 であるから，漂白液残渣は前報[2]に於て述べた廃液処理工程に於て，沈澱池で混合するよりも沈澱池に入る前の濃厚な酸処理濾液に直接少量宛混合するのが妥当である。

Table 6. The effect of pH on rate of reaction between bleeching liquor and Knot-pulp waste.

pH	decrease of free chlorine
11.0	54.6 %
6.6	76.7 %
4.0	88.9 %

総　　括

1. Beater に加えられた遊離塩素は 1 時間の漂白時間中にその 90 % が消費される。
2. Beater での漂白後，洗滌水により溢流する廃液には一般に遊離塩素は 5～20 ppm. の範囲であつた。次にパルプ廃液と混合して放流される時には 1 ppm. 以下であつたから，Beater からの廃水中の遊離塩素は魚類斃死の原因となるとは考えられない。
3. 漂白液製造槽の残渣は一時に放流すれば魚類斃死の原因となる惧れがある，しかしこれをパルプ廃液又はその酸添加処理濾液と混合する事により遊離塩素を除去することは容易であることがわかつた。
4. パルプ廃液と漂白液との反応速度は pH が降る程早くなる。
5. 漂白液残渣は前報の廃水処理工程に於て，処理濾液に直接混合した方が合理的である。

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2) 河辺克己，富山哲夫：“工場廃水の浄化方法に関する研究 III”，日水会誌，巻，号，頁，(1955)，記入を乞う。

工場廃水の浄化方法に関する研究—V.
アルカリパルプ廃液の毒性について

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Studies on Purification of Industrial Waste—V.
On the Nature of the Poisonous Substance in Alkali Pulp Waste

Katsumi KAWABE and Tetuo TOMIYAMA

This paper deals with the estimation of poisonous effect on *Scenedesmus obliquus* and *Carassius auratus* due to presence of waste liquors from caustic soda-digests of the knot and the chips, and further with searching for the poisonous ingredients of the wastes. Results obtained can be summarized as follows :

1) The inhibition of growth of *Scenedesmus* due to the waste from the knot-digest was observed until the waste be diluted 100 times. The inhibition effect was as much decreased as about one-tenth of the original waste on acidifying the warm waste with hydrochloric acid followed by filtration (Figs. 1 and 2).

2) The 24-hr death responses of fish to the wastes from the knot- and the chips-digest were estimated to be at dilutions of 1/800 and 1/90, respectively (Fig. 3). The observation that the fish showed in presence of the wastes a dark coloration and unbalanced state accompanied with an abrupt mad swim, implies that the poisonous substance or subtances will primarily attack the nervous system.

3) The insoluble part obtained by the acid-treatment also showed the poisonous effect to nearly the same extent as the original waste. Most of the poisonous substances in this part could be extracted wish ether. The 24-hr death response to the ethereal extracts of the liquors from the knot- and the chips-digest were estimated to be 170 and 340 p.p.m., respectively (Fig. 3).

4) Taking into account the contents in the knot of pitch abietic acid, pinosylvin, and pinosylvin monomethylether, and data for the death response of the fish to these compounds and also to the autoclaved products of them dissolved in N-NaOH, it will be most probable that pitch abietic acid will be one of the principal compounds responsible for hazard of the waste to aquatic organisms (Fig. 4).

パルプ廃液の毒性については従来種々の研究[1,2,3,4]が行われ，その概略はすでに第一報[1]に記した。著者らは前記アルカリパルプ工場廃液の生物に対する有害物質の本体を明らかにする目的を以て，*Scenedesmus obliquus* の生長阻害作用，並びにヒブナ或いはキンギョ仔魚の生物試験を行い，また亜硫酸パルプ工場の未蒸解ノットから抽出した pitch abietic acid，木材より抽出した pinosylvin 及びその誘導体，及びそれらをアルカリ溶液中で加圧加熱したものについて生物試験を行った。その結果，アルカリパルプ廃液のエーテル抽出物の毒性の本体は，pitch abietic acid であることを認めたので，その結果を報告する。

実　験　之　部

I. *Scenedesmus obliquus* の生長に対する阻害作用

1. 試料並びに実験方法

試料は第一報で用いたもの（knot-pulp waste）と同一のもので，処理試料としては pH 4.5 で塩酸熱処理した時の濾液を用いた。

Table 1 に示す基礎培地を予め乾熱滅菌した径 20 mm の試験管に 4 cc. 宛分注し，更に種々の倍率に

1955 年 12 月 20 日受理

Table 1. Medium of *Scenedesmus obliquus*.

$MgSO_4 \cdot 7H_2O$	0.4	g.
$CaCl_2$	0.02	g.
K_2HPO_4	0.56	h.
Citric acid	0.155	g.
NH_4NO_3	0.2	g.
$FeSO_4 \cdot 7H_2O$	1.0	mg.
KBr	0.01	mg.
KI	0.005	mg.
$MnCl_2$	0.01	mg.
$CuSO_4 \cdot 7H_2O$	0.01	mg.
$CoSO_4 \cdot 7H_2O$	0.01	mg.
$ZnCl_2$	0.01	mg.
$SiO_2(Na_2SiO_3)$	10.0	mg.
Glucose	2	g.
Casein hydrolyzate with acid	1	g.
Casein hydrolyzate with alkali	1	g.
NaCl	0.1	g.
Distilled water	1000	cc.

稀釈した試料 4 cc. 宛を注加したのち，加圧滅菌したものを供試した。之に予め培養した *Scenedesmus obliquus* 2 滴宛を無菌的に接種した後，約 30°C の保温室で螢光燈下に時々振盪しつゝ約3日間培養してその生育状態を観察した。なおこの試験の対照試料としては上記試料の代りに蒸溜水 4 cc. を注加したものを用いた。接種直後と培養3日後のものゝ 420 mμ における optical density を測定し，その差を以て生長量とした。この値は培養条件により一定しないので，対照区の成長量を 100 とした時の各試料の生長量を以て生長比率とし，これにより阻害度を判定した。

2. 実験結果

Fig. 1 に示した結果をみると，原廃液の場合は 40 倍稀釈で全く成長せず，110 倍で対照値に達しているのに対し，処理濾液については 2 倍稀釈では生長しなかつたが，11 倍で 100 の生長比率を示している。何れの場合もこれ以上稀釈すると対照区より反つて成長がよくなつているのは注目に値する。両試料についての成長曲線がよく似ているので，双方を比較すると原廃液の生長阻害作用は処理によつて 1/10 に減少することがわかる。

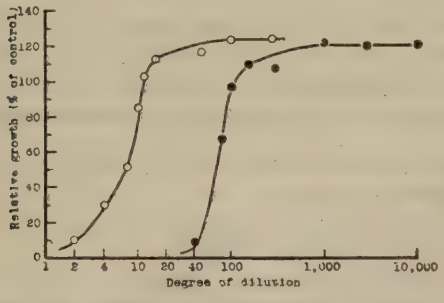

Fig. 1. Inhibition of growth of *Scenedesmus* due to presence of varying amounts of original waste, ●, or the treated waste, ○.

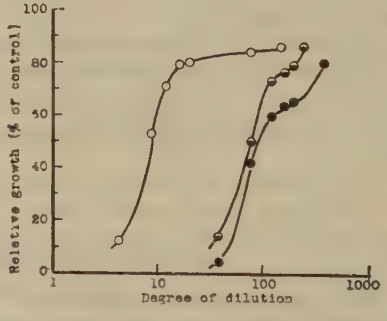

Fig. 2. Inhibition of growth of *Scenedesmus* due to presence of varying amounts of original waste, ●, a diluted and filtrated waste, ◒, or the treated waste, ○.

次に原廃液の場合，150 倍までの稀釈では加圧滅菌する際に培地と反応して多少溷濁するので，滅菌する前に予め培地と共に煮沸して生じた沈澱を濾過したものについて同様の試験を行つたところ，Fig. 2 に示すように僅かに阻害作用に減少がみられた。

II. ヒブナ仔魚に対する原廃液，酸添加処理濾液，及び処理残渣の毒性

1. 試料並びに実験方法

本実験に用いた廃液は第2報の研究に使用した原廃液である。また毒性実験に供したヒブナ仔魚は市販の体長 3.5～5 cm のまだ赤くならない未成熟のものである。実験方法としては予め一昼夜放置した井水を縦 21 cm，横 28 cm，深さ 5 cm の琺瑯引きバットに一定量充し，ヒブナを 4 尾宛放ち，次に充分空気を通じた廃液或いは酸処理濾液を種々の濃度に加えてから観察を開始した。致死の限界はガラス棒で魚体を触れても反応しない場合とした。なお pH は何れも 7 附近に塩酸或いは苛性ソーダで調整した。毒性実験中の水温は 8°C 乃至 14°C であつた。

2. 実験結果

24 時間致死量は knot-pulp waste の場合約 800 倍稀釈濃度，chips-pulp waste の場合約 90 倍稀釈濃度で，赤松チップを原料とした場合よりノットカスを原料とした場合の方が遙かに毒性は強かつた。しかしその酸添加処理濾液についてみると，knot-pulp waste の場合には 4 倍稀釈濃度は全く毒性を示さないが，

chips-pulp waste の場合には約 10 倍稀釈濃度が 24 時間致死量であつた。このことから chips-pulp waste は knot pulp waste にはない毒性を持つていると考えられるが，これは非常に毒性が弱く而も受水区域が酸性化しても沈澱しないので，実際上魚類斃死の原因になる心配はない。

魚の致死状態を観察すると，knot-pulp waste に於て約 2,000 倍，chips-pulp waste で約 200 倍よりも高い濃度では，死ななくても一様に黒変し，平衡を失して時に狂奔旋回運動を繰り返すのがみられ，また死後も鰓を閉ぢていることから神経性毒物の存在が推定される。

酸添加処理時にできる沈澱残渣の懸濁液の毒性については，すでに第三報[6]に於て述べた。

III. 処理残渣のエーテル抽出物の毒性及びその本体

処理残渣はベンゾールに不溶，アセトンには全部溶け，エーテルには一部溶解した。原廃液よりは酸性，アルカリ性何れに於てもエーテル抽出は可能であつたが，強アルカリ性では抽出困難であつた。原廃液よりのエーテル抽出物量は knot-pulp waste で約 2.1 %，chips-pulp waste では約 1.3 % であり，固型物当り夫々 17.1 %及び 7.3 %に相当した。

knot-pulp waste を酸添加処理した時，得られる沈澱をエーテル抽出した後の残渣を苛性ソーダで溶解したものについて，ヒブナ仔魚に対する毒性試験を行つたところ，殆んど毒性を示さなかつた。このことから，knot-pulp waste の毒物のほとんどはエーテル抽出物中に含まれていると考えられる。chips-pulp waste の場合は酸添加処理後の濾液にも多少の毒性が残ることは既に述べたが，この点は knot-pulp waste の場合には濾液にエーテル抽出物が存在しないのに対し，chips-pulp waste の場合には濾液中にもエーテル抽出物が残る事実と符合している。

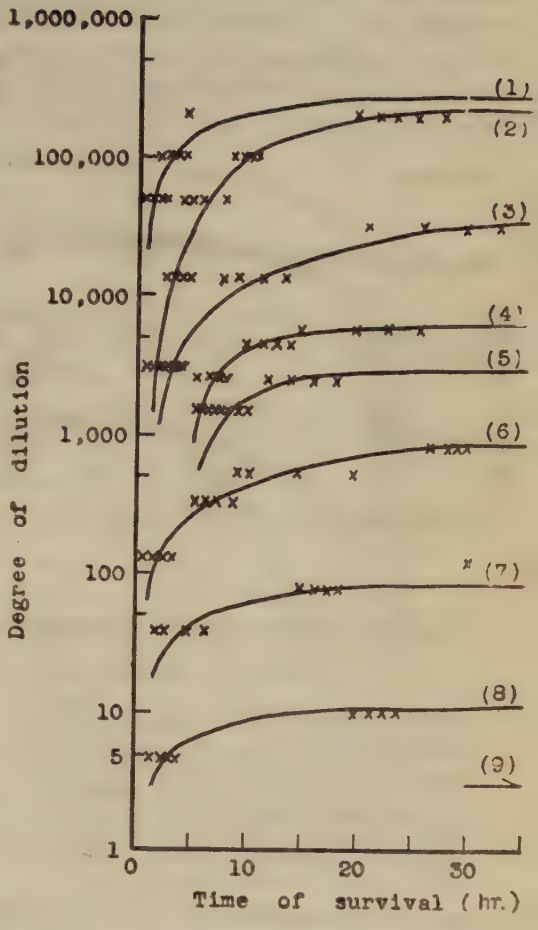

Fig. 3. Change in time of survival of *Carassius auratus* with change in concentration of the following samples; (1) pitch abietic acid, (2) rosin, (3) resene, (4) ether extract of knot-pulp waste, (5) ether extract of chips-pulp waste, (6) knot-pulp waste, (7) chips-pulp waste, and (8) treated filtrate of chips-pulp waste. No motality was observed in (9), treated filtrate of knot-pulp waste.

沈澱残渣をエーテル抽出し，エーテルを除去したのち少量のアルコールに溶かし，これを曝気した井水に混和したものについてヒブナ仔魚に対する毒性を見た。試水は多少濁つているが，その致死濃度は Fig. 3 に示すように，knot-pulp waste のエーテル抽出物は chips-pulp waste に比べると約2倍の毒性を示している。

次にエーテル抽出物中の有害物質の本体について更に吟味してみた。パルプ原料である赤松並びに亜硫酸工場の未蒸解ノットのエーテル抽出物について分析した結果から，本試験に於けるパルプ廃液特に knot-pulp waste の有機性毒成分の大部分は樹脂酸及びその関連物質と考えてよいと思われる。黒木氏ら[7]は亜硫酸パルプ工場の未蒸解ノットのエーテル抽出物から結晶成分を得，この物質が $C_{20}H_{30}O_2$ の分子式をもつ abietic acid 類の樹脂酸であることを確かめ，pitch abietic acid と命名している。同氏の研究[7]によればノットのエーテル抽出物の約 90 % はこの物質であるから，knot-pulp waste のエーテル抽出物の大部分はこ

の物質と推察される。よつて pitch-abietic acid，エーテル抽出物中の中性物質（resene）及び rosin（松脂の乾溜成分）について生物試験を行つた。試料は何れも少量のアルコールに溶かした後，充分曝気した井水に混じた。この試験にはヒブナ仔魚が得難かつたので，前供試魚と同一池で飼育した同程度の体長をもつ金魚の仔魚を用いたが，予備試験の結果該毒物に対する抵抗性は何れもほとんど異ならなかつた。生物試験の結果は Fig. 3 に示す通りであつて，いずれも毒性は強いが，pitch abietic acid が最も強い毒性を示した。

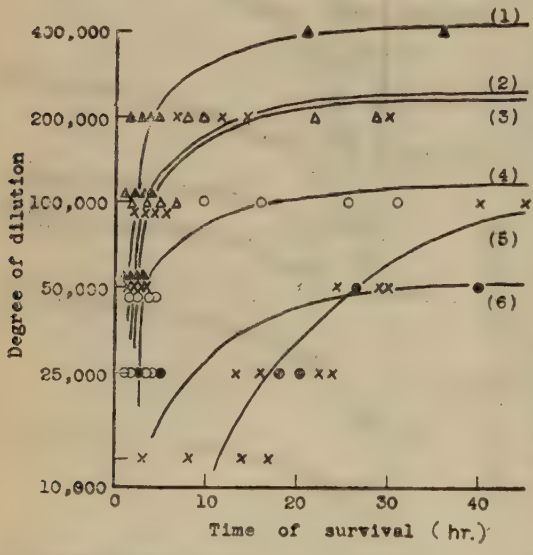

Fig. 4. Showing that a process of autoclaving in N-NaOH under pressure of 4.5kg /cm² did affect the time of survival of *Carassius auratus* in pitch abietic acid (curve 5, with ; curve 3, without autoclave) and pinosylvin (curve 4, with ; curve 6, without autoclave) while it did not affect in pinosylvin monomethyl ether (curve 2, with ; curve 1, without autoclave).

なお，木材中の微量成分として知られている Pinosylvin を得たので，pinosylvin 並びに pinosylvin monomethyl ether について同様の生物試験を行い Fig. 4 の結果を得た。pinosylvin が強い抗菌性をもつことはすでに浮田氏ら[8]によつて明らかにされているが，金魚に対する毒性は pinosylvin で 7 p.p.m.，pinosylvin monomethyl ether の場合には pitch abietic acid とほとんど異ならず約 5 p.p.m. が 24 時間致死濃度であつた。

次にパルプ製造工程中に digester では 8 kg/cm² で約 2 時間蒸解するので，木材中のこれらの成分は変化を受けると思われる。そこで，pitch abietic acid，pinosylvin，及び pinosylvin monomethyl ether を各々 1 規定苛性ソーダ溶液と共に試験管に入れて綿栓後オートクレーブに入れ，実際の温度よりは低いが 4.5 kg/cm² で 2 時間加圧加熱した。それらのものについて金魚に対する毒性試験を行つた結果は Fig. 4 に示すようである。即ち，pinosylvin monomethyl ether の場合を除いて，pinosylvin 及び pitch abietic acid の場合は，何れも加熱処理後は毒性が減少するのがみられ，24 時間致死量は前者で約 1/4，後者では 1/5 に減少し，24 時間致死量はいずれも約 20 p.p.m. であつた。しかし pinosylvin は原材中の含有率が少いことから考えて毒性の主体をなすものとは考えられない。依てアルカリパルプ廃液を酸添加処理して沈澱する部分の毒性の本体は恐らく pitch abietic acid であろうと推論される。

総　括

1. *Scenedesmus obliquus* に対する毒性は，knot-pulp waste の場合約 100 倍，酸処理濾液では約 10 倍に稀釈すれば成長阻害作用がなくなる。

2. ヒブナ仔魚に対しては約 10°C 前後で knot-pulp waste では約 800 倍，chips-pulp waste では約 90 倍の稀釈濃度が 24 時間致死濃度であつた。また処理濾液の場合には前者についてはほとんど毒性を示さぬが，後者では僅かに毒性を残した。

3. 酸添加処理によつて出来る沈澱残渣の懸濁液も原廃液と同様の毒性を示した。

4. 廃液中の毒物は体色の変化，魚体平衡の失調，狂奔游泳等の観察結果から神経性毒物と思われる。

5. 廃液を酸処理したとき得られる残渣中の毒物の大部分はエーテルによつて抽出され，knot-pulp waste 及び chips-pulp waste の各エーテル抽出物の 24 時間致死量は夫々 170 p.p.m. 及び 340 p.p m. であつた。

6. 亜硫酸パルプ工場の未蒸解ノットからエーテルで抽出した成分，その成分の毒性及びこれらのアルカリ蒸解処理後の毒性残存程度から判断すると，アルカリパルプ廃液の有機性有毒成分の本体は pitch abietic

acid と考えられる。純粋な pitch abietic acid の 24 時間致死濃度は 5 p.p.m. であつた。

最後に本研究について種々御助言を賜つた九大農学部の西田乾二教授並びに渡部常樹助教授に深謝し，またアビエチン酸その他貴重な試料を提供して戴いた九大林産化学教室黒木董氏，ヒノシルビンの提供を戴いた香川大学の堀克美教授，また生物試験に当り種々便宜を与えられた津田厚氏に深く感謝する次第である。

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水産醱酵食品の酵母に関する研究（第6報）

無胞子酵母の培養的並びに生理学的性質について

錢　谷　武　平

Yeasts occurring in fishery-fermentation products. Part 6

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Buhei Zenitani

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先に無胞子酵母の形態学的特徴について報告[1]したが，本報においては分類学的研究特に培養的並びに生理学的性質と醱酵食品の製造・保蔵などに関係のあるこれら酵母の発育条件，耐塩力等について検討した結果を報告する．

一般菌学的性質

I. 無胞子酵母の研究方法

無胞子酵母の分類には Will 以来諸氏の研究があり，その内 Lodder (1934)[2]の分類が最も重要視されたが *Mycotoruloideae* 亜科は未完であつた．従つて著者は本亜科の分類には Dodge[3]，Ciferri & Redaelli[4]及び Martin[5]を参考にし Lodder の方法を基にして研究を進めたが，その後 Skinner (1948)[6]や完結した Diddens & Lodder (1942)[7]の研究をも参考にした．又最近 Lodder & van Rij (1952)[8]が酵母菌全般についての研究を発表したのでこれにより若干の補足を行つた．本研究は単に分類のみを目的としなかつたので諸種の試験法を検討して結局下記の如き研究方法に従つた．供試菌は前に報告した8菌株である．

(A) 培養的性質の試験法

1) 液体培養 :— 麦芽汁 Bllg. 15°, 25°C.
2) 劃線培養 :— 麦芽汁寒天 Bllg. 12°, 25°C, 7日.
3) 穿刺培養（溶膠性）:— 麦芽汁膠 Bllg. 10°, 20% 晒膠.
4) 巨大集落 :— 同上培養基.

1～4）の各試験は真正酵母[9]と全く同様に行つた．Lodder & van Rij は穿刺培養及び巨大集落の特徴を標式に採用していないが著者は既往の研究と比較検討する必要から実施した．

また Sabouraud medium を用いて液体，斜面及び巨大集落の特徴を確めた．

(B) 生理学的性質の試験法

1) 各種糖類醱酵試験 :— Lindner 小醱酵法.

2) 各種糖類同化試験 :— Lodder は非醱酵性菌株のみにこの試験を行つたが，其後更に酵母菌全般に広く採用された．基本培養基は $(NH_4)_2SO_4$ 0.5%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% 及び洗滌寒天 2% を用い Auxanograph 法によるかあるいは供試糖 2% を加えて斜面とし発育の有無を 25°C に培養して観察したが後者の場合は無糖のものを対照とした．葡萄糖，ガラクトーズ，蔗糖，麦芽糖及び乳糖を用いて試験した外，別にキシローズ，アラビノーズ等のペントーズをも試験した．

3) 糖類生酸試験 :—

4) 窒素化合物同化試験 :—

5) 酒精同化試験 :—

6) 皮膜生産試験 :— 3～6は真正酵母の試験法に同じ．

7) エステル生産試験 :— この試験は *Hansenula* 属の不完全期に相当すると認められる *Candida* 属の識別に有効である．酵母エキス（パン酵母 200g に水 1*l* を加えて 120°C において15分間加熱後濾過）に葡萄糖 5% を加えて 20cc 宛を 50cc 容三角コルベンに分注し殺菌後，新鮮培養から接種し 25°C に1週間培養してから検査した．

8) 澱粉（類似物質）生成試験 :— Lodder & van Rij と Wickerham[10]との試験方法は多少異るが前者に従い下法のようにした．基本培養基は $(NH_4)_2SO_4$ 0.1%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, 葡萄糖 1% 及び寒天 2.5% から成り稀塩酸で pH 4.5 に調整した．平板培養には酵母エキスを1平板当り2滴加えた．25°C に2週間培養した後ルゴール液を加えて澱粉質生成の有無を検査した．

9) リトマス牛乳培養 :— 2種の培養基，即ち a) 脱脂乳 10cc に 1% リトマス液を 1cc 加えたもの，b) a に5% 乳酸石灰液を1cc 追加したものを用意した．25°C において30日間培養し凝固，ペプトン化及び色調の変化を観察した．

10) 脂肪分解試験 :— Lodder & van Rij は *Candida lipolytica* 等の少数菌株のみに牛脂分解試験を行つているが著者は次の如くトリブチリンを基質とし乳濁油脂の透明化により判定する方法を採つた．葡萄糖 0.5%, ペプトン 0.5%, 魚肉エキス 0.3%, トリブチリン1% 及び寒天 1.5% の基本培養基を加熱溶解し強く振盪して乳濁させてから小試験管に分注し加圧殺菌した．直立固化してから寒天表面に一様に接種し 25°C に 10日間培養して透明層の深さを測り強弱を判定した．またオリーブ油と牛脂を基質として Peter & Nelson[11]の改変培養基を用いて試験した．

11) 発育最適 pH :—

12) 発育最適温度 :—

13) 死滅温度 :—

14) 食塩抵抗発育試験 :— 11～14) の試験法は真正酵母の場合に同じ．

II. 試 験 結 果

(A) 1) 液体培養 2) 劃線培養及び穿刺培養の特徴はそれぞれ Table 1, 2 及び3に

Table 1. Fluid culture at 25° C (Wort, Bllg. 15°).

Strain	Note
D_1	Turbid after 24 hours, and a little sediment after 6 days.
K_1	Clear after 3 days, slight ring and a little sediment after 6 days. Ring and floating thick pellicle after 10 days.
M_1	Some islets and ring after 3 days, a light yellow ring and slightly turbid after 6 days.
M_2	Turbid after 3 days, becoming clear with a sediment after 6 days. Ring formed slightly after 10 days.
N_2	Clear, a little sediment after 3 days. Greyish white ring after 10 days.
O_2	Clear, a little sediment after 3 days. Greyish white ring after 10 days.
S_1	White powdery pellicle, turbid slightly and a moderate sediment.
S_5	Ring formed slightly and a little sediment after 6 days.

Table 2. Streak culture at 25°C (Wort agar, Bllg. 12°).

Strain	Note
D_1	White porcelaneous, smooth, raised slowly and entire edges.
K_1	Greyish white, glistening, flat and entire edges.
M_1	Greyish white, sebaceous, smooth generally, raised slowly and entire edges.
M_2	Milky white, sebaceous, smooth, flat and undulate.
N_2	Greyish white, waxy, flat, slightly striped at the border and the margin slightly sinuous.
O_2	Greyish white, sebaceous, smooth, raised slowly and entire edges.
S_1	Dry, brownish grey, many folds at the surface, raised generally and irregular edges.
S_5	Moist, greyish white, waxy, generally raised, finely granular, slightly striped at the border and slightly sinuous edges.

Table 3. Stab culture with wort gelatin.

Strain	Liquefaction (Cult. days)	Note
D_1	−(50)	Circular with radial sectors on the surface.
K_1	−(70)	Sebaceous, slowly raised, yellow at the center. No growth in the stab line.
M_1	−(70)	Dull, yellow and raised slowly.
M_2	−(70)	Greyish white, glistening and flat.
N_2	−(70)	Greyish white and poor growth.
O_2	−(70)	Greyish white and raised slowly.
S_1	+(30)	White powdery, irregular, dark brown at the center and growth in the stab line (depth of growth: 8mm.).
S_5	+(30)	Greyish white, many folds at the surface and merged growth into the stab line.

Table 4. Form and texture of giant colonies of asporogenous yeasts. (Wort gelatin).

Strain (days)	Form (dia. mm.)	Color	Elevation and crater	Concentric ring	Radial sector	Edges
D_1 (70)	Circular. (9)	Greyish white, sebaceous.	Generally raised. Indistinct crater.	None.	None.	Entire.
K_1 (60)	Irregular. (19)	Light yellowish grey at the center and greyish white, waxy at the border.	Raised at the centre only, double crater form.	Indistinct some rings.	Many at the border.	Undulate.
M_1 (60)	Circular. (12)	Ditto.	Generally flat.	Indistinct rings.	None.	Entire.
M_2 (60)	Irregular. circular. (19)	Ditto.	Generally flat, raised slowly. Small crater.	Ditto.	Many at the border.	Undulate.
N_2 (60)	Circular. (13)	Greyish yellow at the center, greyish white at the border.	Raised slowly.	Indistinct some rings.	Many folded lines.	Ditto.
O_2 (60)	Circular. (11)	White waxy at the center, grey at the border.	Raised at the center only.	Indistinct many rings.	Some at the border.	Ditto.
S_1 (70)	Irregular. (21)	Dry, white. Many folds at the surface.	Raised generally, and merged irregularly at crater.	One.	Indistinct.	Irregular.
S_5 (70)	Irregular. (12)	Dry, grey at the center and white at the margin.	Raised at the center and small crater.	Wrinkled at the center.		The irregular margin, surrounding by pseudomycelia.

表示したように醱酵性は本試験ではほとんど認められなかつた．5％葡萄糖ペプトン水においても醱酵性はほとんど見られず*僅に数菌株が Lindner 小醱酵法においてのみ弱い醱酵性を示すに過ぎなかつた．塩辛中の無胞子酵母は概して醱酵型でなく酸化的分解を行うものと考えられる．溶膠性はほとんど認められないが S_1 及び S_5 は膠内に没入して特徴のある発育を示した．

4）巨大集落：— 供試 *Candida* 属酵母のうちに Rough 集落が現れることがあつた．既に *Candida albicans*（Robin）Berkhout[8]で観察されているが Smooth 集落に較べ細胞は Mycel 状に伸長し，また巨大集落は古くなると R 部と S 部では細胞形が異り R 部には一層伸長した細胞が見られた．Lodder に依れば累代培養によつて S 型から R 型へ移行すると．

（B）1～9）の試験結果は Table 5 に一括表示した．

Table 5. Physiological properties of asporogenous yeasts.

Test	Strain	D_1	K_1	M_1	M_2	N_2	O_2	S_1	S_5
1-3) Sugar-fermentation and assimilation. Acid production.	Glucose	GAS	-As	-As	GAs	GAs	-As	-A	GAS
	Galactose	? - -	- - -	-A-	-A-	-A-	-A-	- -	- - -
	Saccharose	? AS	-A-	-As	-As	-As	-A-	- -	-A-
	Maltose	-As	-A-	-A-	-As	-As	-A-	- -	-A-
	Lactose	- - -	- - -	- - -	- - -	- - -	- - -	- -	- - -
4) Assimilation of nitrogen-compounds.	KNO_3	−	−	−	−	−	−	−	−
	$(NH_4)_2SO_4$	+	+	+	+	+	+	+	+
	Urea	+	+	+	+	+	+	+	+
	Asparagin	+	+	+	+	+	+	+	+
	Peptone	+	+	+	+	+	+	+	+
5) Assimilation of ethyl alcohol.		+	+	−	+	+P	+	−	+
6) Pellicle formation in wort.		+R	−	+R	−	−	−	+	−
7) Ester (Ethyl acetate) formation.		−	−	−	−	−	−	−	−
8) Starch-like substance production.		−	/	/	−	−	−	/	−
9) Litmus milk culture.		blue	/	/	blue	blue	n. c	/	n. c
Ditto (with calcium lactate).		n. c	/	/	blue	n. c	n. c	/	n. c

Note: G … gaseous fermentation, A … assimilation, S or s … acid. P … Pellicle, R … ring and n. c … no visible change.
The methods employed are the same as described in the case of true yeasts, unless otherwise stated.

無胞子酵母の醱酵性は麦芽汁あるいは 5％ 葡萄糖ペプトン水においても認められず Lindner 小醱酵法で認知される如く極めて弱く *Debaryomyces* 属の醱酵能に類似するようである．葡萄糖，麦芽糖及び蔗糖は多くの菌によつて同化されたが乳糖は全く同化されなかつた．前に[12]*Candida* sp. No 2（N_2）はペントーズ，マンニットをよく同化し海

* 葡萄糖 2.0％，肉エキス 0.3％，ペプトン 1.0％，食塩 0.5％（pH = 7.2）の場合に 30 °C で D_1，N_2 及び M_2 は弱いガス発生を見た．

藻を原料として酵母製造に有用であることを報告したが O_2 も同様にペントーズを同化し S_1 は葡萄糖のみを同化したにすぎなかつた.

窒素化合物の同化性は真正酵母の場合と全く同様で，酒精同化性も S_1 と K_1 を除く全菌株に認められた．皮膜生産は麦芽汁 B11g. 12° と Sabouraud 液とでは違い後者の場合は M_2, N_2 両菌に薄膜の形成を認めた.

エステル形成試験の結果，臭気はむしろ酸臭又は味噌臭に近く *Hansenula* 属の如き顕著なエステル香は感知されなかつた．澱粉類似物質生成試験の結果はルゴール試薬でグリコーゲン形成を認めたものもあつたが澱粉質形成はいずれも認められなかつた.

Table 6. The lipolytic activities on tributyrin agar.

Strain \ Cult. days	4	8	10
D_1 ……(*Candida* species)	±	+	+
M_2, N_2. X_2 ……(*Candida* species)	+	++	++
S_5……(*Candida* species)	−	−	−
O_2……(*Torulopsis* species)	−	−	−
Debaryomyces hansenii…… (R_4)	+	++	++
Debaryomyces kloeckeri…… (A_2 and S_4)	+	++	++
Debaryomyces nicotianae…… (P_2)	+	++	++
Debaryomyces nicotianae var. *salsus*…… (C_2)	+	++	++
Debaryomyces species V_1	+	++	++
Saccharomyces rouxii…… (N_1 and Q_2)	−	±	+

Medium: Glucose 0.5%, peptone 0.5%, fish-extract 0.3%, tributyrin 1% and agar 1.5%. These constituents were emulsified, poured into small test tubes and allowed to harden. The activity was measured by the depth of clear zone.
Depth of clear zone: ++ 4 mm <, ++ 3 mm <, + weak and − negative.

10) 無胞子酵母の脂肪分解能は従来専ら牛脂を用いて *Candida*, *Trichosporon* 属の小数菌株に認められているがトリブチリン基質では *Candida* 属の大部分と同時に試験した *Debaryomyces* 属の全菌株に分解力を認めた．なお牛脂とオリーヴ油の場合は分解を認めた菌株はあつたがいずれも微弱であつた.

11～13) の結果は Table 7 に表示したが発育至適 pH は概ね 5.0～7.0 で真正酵母の 4.1～6.0 よりも幾分高く D_1 のみは 3.3～5.0 であつた．発育温度並びに死滅温度の関係は真正酵母におけるとほぼ同様であるが M_2 と N_2 のみは 38～41°C においても発育することは注目に値する．また K_1 は 50°C, 10分間の加熱で死滅し塩辛酵母中で最も低い値を示した.

14) 塩辛酵母のうち真正酵母は食塩を 15% 含有する培養基中でもよく発育したが無胞子酵母は一般に耐塩力がやや劣る．*Mycoderma* 属 (*Candida mycoderma* Lodder et van Rij) もまた Mrak & Bonar[13] の研究では *Debaryomyces* 属より耐塩力が弱く其他含塩食品から分離された *Candida krusei* (cucumber brine)[14], *Torulopsis uvae* var. *miso* Mogi 及び *Pseudomonilia* (共に味噌[15]から分離され Lodder & van

Table 7. Optimum-pH and -temperature of growth, and thermal death point.

Strain	Growth pH									Opt. pH	Growth temp.					Opt. temp.	Death temp.
	2.0	3.0	3.3	4.1	5.0	6.0	6.3	7.2	7.6		21～23	25	30	35	38～41		
D_1	−	＋	＋	＋	＋	＋	＋	＋	＋	3.3～5.0	＋	＋	＋	−	−	25～30	55°C, 10 min.
K_1	−	−	＋	＋	＋	＋	＋	＋	＋	6.0～6.3	＋	＋	＋	±	−	25	50
M_1	−	−	＋	＋	＋	＋	＋	＋	＋	6.3～7.2	±	＋	＋	±	−	25～30	55
M_2	±	＋	＋	＋	＋	＋	＋	＋	＋	4.1～5.0	＋	＋	＋	＋	＋	30～35	60
N_2	−	＋	＋	＋	＋	＋	＋	＋	＋	5.0～6.0	＋	＋	＋	＋	＋	30～35	60
O_2	−	−	±	±	＋	＋	＋	±	±	6.0	−	＋	＋	−	−	30	55
S_1	−	−	−	＋	＋	＋	＋	＋	＋	6.0～7.2	＋	＋	＋	−	−	25～30	55
S_5	−	−	−	＋	＋	＋	＋	＋	＋	5.0～7.2	＋	＋	＋	±	−	30	60

Table 8. The results of salt-tolerant test.

Strain	NaCl g/100 cc						Note
	0.5	5	10	15	20	25	
D_1, M_2	＋	＋	＋	＋	±	−	Turbid at 0.5—10, ring at 5—10 and slight sediment at 20%.
K_1	＋	＋R	＋R	＋	±	−	Turbid at 0.5—10, slight ring at 5—10, sediment decreasing due to salt concentration.
M_1	＋R	＋R	＋R	＋r	±	−	Turbid at 0.5—15, ring at 0.5—10. Slight ring and moderate sediment at 15%.
N_2	＋r	＋r	＋r	＋	±	−	Turbid at 0.5—10, clear at 15—20 and abundant sediment at 0.5%.
O_2	＋r	＋r	＋	＋	±	−	All clear, slight ring at 5 and sediment at 0.5—10.
S_1	＋P	＋P	＋P	＋P	＋	±	Light yellowish white, dry heavy folded pellicle at 0.5—15, and slight sediment. Small islets at 20—25 and a little sediment at 20.
S_5	＋Pr	＋Pr	＋Pr	＋p	＋	±	Pellicle at 0.5—15, slight ring at 0.5, ring and abundant sediment at 5—10. Thin pellicle and a little sediment at 15—20.

Note: R…ring, P…pellicle.

Rij の分類では *Candida* 属と考えられる）も耐塩力は概ね 10% 食塩であつて食塩濃度の低い製品に分布するものと思う.

要　　約

塩辛酵母の内，無胞子酵母8菌株の培養的並びに生理学的性質について検討した結果を記載した.

醱酵性は全く欠除するか微弱で *Debaryomyces* 属の醱酵能に類似し，その他窒素化合物同化性，酒精同化性も真正酵母のそれと大差はなかつた．特に脂肪分解能はトリブチリ

ンを基質とする場合可成り強く，同時に試験した *Debaryomyces* 属の全菌株が強く分解したことは注目に値する．発育の最適 pH は 6.0～7.0，温度は 30°C 前後で耐塩力は真正酵母よりもやや低いようであた．

概言すれば無胞子酵母の一般菌学的性質は塩辛中の *Debaryomyces* 属のそれに類似するようで，少くとも塩辛中の *Candida* 属酵母は *Saccharomyces* 属や *Hansenula* 属の不完全期のものではないと思われる．

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Résumé

The present study was designed from the taxonomic and practical standpoint to secure the general features of asporogenous yeasts, whose morphological characteristics had already been reported.

Further observations on the cultural characteristics are described in Tables 1～4. The physiological characteristics are listed in Table 5 summarily, which brought out the fact that the test-yeasts dissimilate sugars rather oxidatively than fermentatively, i. e. gaseous fermentation was not observed in the liquid cultures but some strains showed it by Lindner's method only, and no remarkable difference from the true yeasts was observed on the assimilabilities of

nitrogen compounds and ethyl alcohol. Most of the yeasts tested showed the lipolytic abilities when tributyrin agar was used as substrate (Table 6). Their growth-pH, -temperature and thermal death points are shown in Table 7. The optimum pH of their growth (5.0～7.0) is comparatively higher than in the case of true yeasts (4.0～6.0). The salt tolerance was apparently lower than in the case of true yeasts (Table 8).

Generally speaking, the properties of asporogenous yeasts in "Shiokara" seem to have some resemblances to those of *Debaryomyces* species, and these *Candida* species, at least in "Shiokara", are not considered belonging to an imperfect stage of *Saccharomyces* or *Hansenula* species.

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銭　谷　武　平

Yeasts occurring in fishery-fermentation products. Part 7
A taxonomic study of asporogenous yeasts

Buhei Zenitani

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Yeasts occurring in fishery-fermentation products. Part 7
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緒　　言

先に塩害酵母中の真正酵母は主として *Debaryomyces* 属と *Saccharomyces* 属からなり味噌・醤油酵母に深い関係のあることを確め,[1] 更に無胞子酵母の一般菌学的性質を検討しこれらの内には *Saccharomyces* 属や *Hansenula* 属の無胞子世代と考えられるものはなく，むしろ *Debaryomyces* 属の生理学的性質に酷似することを報告した.[2]

本報では無胞子酵母の分類所属を検討し *Torulopsis* 属, *Cryptococcus* 属と *Candida* 属に入ることを明らかにしたので，これらの酵母の分類学的位置及び類縁関係等について報告する.

無胞子酵母の分類と類縁関係

I. 無胞子酵母の種類同定について

無胞子酵母の分類には多数の研究があり最近 Lodder & van Rij (1952)[3] と医学分野では Skinner 等 (1948)[4] の分類が公刊されたが両者の間には著しい相違が見られる. Lodder & van Rij は Skinner 等の *Cryptococcoidcae* (Diddens & Lodder の *Torulopsidoideae*), *Cryptococcus* (*Torulopsis*) 等の名称など若干の事項は採用している. 著者は既報の如き研究方法をとつたので本報においては Lodder & van Rij の分類検索に従つた.

供試酵母は概ね *Torulopsis* 属と *Candida* 属に入るが Skinner[5] が述べた如く *Candida* 属の分類は幾多の変遷を経て，*Torulopsis* 属との識別も必ずしも明確ではないようである. *Candida* 属は厳密には伸長細胞の集合体を以て特徴とし仮菌糸の形成はこれが変移態の場合に判定が困難でかつ仮菌糸形成は外部要因に影響され易い. 即ち試験方法の適否，培養懸により *Candida* 属が *Torulopsis* 属に分類される懸念のあることは *Torulopsis utilis* (Henneberg) Lodder が嫌気条件で仮菌糸を形成することが Fink や Windish によつて認められ *Candida utilis* (Henneberg) Lodder et van Rij とされ，又従来の *Mycoderma* 属は試験法の不適により仮菌糸形成を看過されてい

たのがその形成を確められるに至つて *Candida mycoderma* (Reess) Lodder et van Rij と変更された例もある. 他方 Connell & Skinner[6] は仮菌絲形成をしなかつた 131 株を Sabouraud 寒天で保存後に再試の結果 71 株が仮菌絲を形成し *Cryptococcus* 属* が *Candida* 属に degenerate する事実を認め *Candida pulcherrima* は normal あるいは rough form によつて孰れかに分類される例とした. 又 Lodder & van Rij は無胞子酵母の一部は少くとも真正酵母の不完全期のものと考え *Saccharomyces* 属の不完全期のものは *Torulopsis* 属あるいは *Candida* 属に入るものとしている.

かように両属の区別は明確でないようで所属決定は専ら仮菌絲形成試験に俟たねばならない現状である. 著者は従来の方法以外に新に米糠汁穿刺法を考案して仮菌絲形成試験において好結果を得たが, 仮菌絲の発育は供試菌によつては結果が一定しないこともあつたので著者は各種試験法を施行しその内いずれかの試験で仮菌絲態の発育を示したものは *Candida* 属とした.

なお *Candida* 属の命名法に関し応用的見地から最近小林氏[7,12] は Lodder & van Rij の**種**を**亜属**として更に種を区別されているが著者は本属菌の分類が従来の多くの**属**並に**種**を整理統合されつつある傾向から考え[5] 分類学的見地におけるかぎり Lodder 等の命名法に従つた.

供試8菌株の分類学的所属位置を表示すると次の如くであつてその類縁関係については以下にしるす.

The identified species of asporogenous yeasts by the classification of Lodder & van Rij (1952).

Family *Cryptococcaceae*
Subfamily *Cryptococcoideae*

Genus 2. *Torulopsis* Berlese
Torulopsis famata (Harrison) Lodder et van Rij ..... M_1 and O_2

Genus 5. *Candida* Berkhout
Candida mesenterica (Geiger) Diddens et Lodder ..... K_1
Candida parapsilosis (Ashford) Langeron et Talice ..... M_2 and D_1
Candida parapsilosis var. *intermedia* van Rij et Verona ..... N_2
Candida polymorpha nov. spec. ..... S_5

An undetermined species: *Cryptococcus* species ..... S_1

II. 無胞子酵母の分類と類縁関係

Torulopsis Berlese

1) *Torulopsis famata* (Harrison) Lodder et van Rij ..... M_1, O_2.

* Skinner は *Torulopsis* 属名を命名法上正しくないとして上記属名を用いている. Lodder et van Rij の分類における *Cryptococcus* 属とは区別さるべきものである.

両菌株は仮菌絲を形成せず *Torulopsis* 属に該当する．M_1 は *T. famata* にほぼ一致するがガラクトーズを同化せず，かつ僅かに島嶼を形成する点でやや異る．ガラクトース同化性は両氏の分類に於て必ずしも決定的な特徴となつていない種類もあり（同一種において＋or－）原著 *Mycotorula famata* の記載では薄膜を形成する．これらは種を識別する本質的な相違とは認め難く *T. famata* に包含されるべき菌株であると思う．Diddens & Lodder[8] の検索に従うと *Torulopsis minor* (Poll. et Nann.) Lodder と *T. uvae* (Poll. et Nann.) Lodder に近縁で *T. uvae* var. *miso* Mogi[9] もあるが，後2者は *Candida zeylanoides* (Cast.) Langeron et Guerr 及びその変種となり M_1 とは異る．

O_2 は Sabouraud 寒天上においては *Pityrosporum* 属を想起させるような巾広い出芽をするが本属菌は麦芽汁又は同寒天に発育が貧弱であるに比し O_2 はよく発育するので *Torulopsis* 属とするのが妥当であつて M_1 と同様に *T. famata* に殆ど全く一致した．

Torulopsis famata は Lodder & van Rij によれば *Debaryomyces kloeckeri* Guilliermond et Péju の不完全世代と見なされている．

Candida Berkhout

2) *Candida mesenterica* (Geiger) Diddens et Lodder ……K_1

K_1 は形態的特徴，殊に先端の尖つた長細胞があり *Brettanomyces* 属とも考えられるが醱酵性と硝酸塩同化能を欠くので *Candida* 属に該当し *Candida mesenterica*（旧名 *Pseudomonilia mesenterica* Geiger）に近縁である．西村氏[10]は *Pseudomonilia Miso* A 及び B を，茂木氏[11]は *Pseudomonilia miso* Mogi をそれぞれ味噌から分離した．*P. Miso* A の葡萄糖醱酵性は疑しく芝木氏の *Pseudomonilia* 属菌が単糖以外に麦芽糖，蔗糖及びトレハローズ（弱）を醱酵する点，前者と異り新種とされた．従来 *Pseudomonilia* 属として報告された菌種の醱酵性は疑わしく Lodder 等はその理由を Lindner 小醱酵法の使用に帰し彼等は Einhorn 管を使用し醱酵性を認めていない．従つて本属菌の Lindner 法による醱酵能は検討を要するが著者は K_1 が同法でも全く醱酵性を示さず *Candida mesenterica* に極めて近似するものと認め，なお醱酵性以外の点では前記の味噌酵母にも近い．

3) *Candida parapsilosis* (Ashford) Langeron et Talice ……M_2, D_1

4) *Candida parapsilosis* var. *intermedia* van Rij et Verona……N_2

D_1, M_2 及び N_2 は共に仮菌絲と Blastospore を形成し Arthrospore を形成せず *Candida* 属に該当し *Candida parapsilosis* 及びその変種 *intermedia* に最も近縁である．糖類醱酵性において僅かに相違するが標式内の差にすぎないようで，ただ N_2 はガラクトーズを醱酵しない点では小林氏[12]の分離したマンノーズとガラクトーズを醱酵しない *Candida* (*parapsilosis*) *vinealis* Kobayashi に近い．

M_2 は *C. parapsilosis* にほとんど全く一致し N_2 は仮菌絲細胞中に屈曲した細胞があり酒精同化試験では産膜し変種 *C. parapsilosis* var. *intermedia* に一層近いものと思われる．D_1 は生理学的一般性質の内，糖類に対する作用は全く一致したので形態学的には *Candida melini* Diddens et Lodder に近いようであるが生理学的性質を重視し

C. parapsilosis に近縁のものと認める.

5) ***Candida polymorpha*** nov. spec. ……S_5

本菌の細胞形は極めて変異に富み多形態を示し，その仮菌絲の発育は“Blastodendrion”型に属する．楕円形，腸詰形又は円錐型の先端のやや尖つた形等の細胞からなり弱酸酵性を示しているが硝酸塩を同化しない点から *Brettanomyces* 属とは異り *Candida* 属に該当する.

Fig. 1. ***Candida polymorpha*** nov. spec.

A, B. Vegetative cells on wort agar (4,7 days at 25 °C) C, D, E. and F. Pseudomycelia and blastospores (C…Carrot agar, D…Saubouraud agar, E…Lodder's medium and F…Rice bran agar stab culture). ↑ show cross formation.

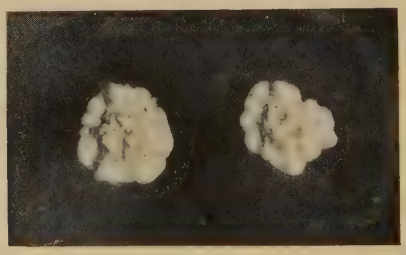

Fig. 2. Giant colony of ***Candida polymorpha*** nov. spec.

本菌の仮菌絲細胞と Blastospore の細胞形の相違は少く，これら細胞は概ね中央部が膨脹した形を示し時には膨脹部が端部に見られる．分枝又は Blastospore の着生は仮菌絲細胞の先端部のみならず中央附近でも行われる．所謂 *Nectaromyces* 型の細胞即ち *Candida reukaufii* の十字架形成は仮菌絲細胞の端部から両方に伸長形の Blastospore が突出してT又は Y 字形を示して Cross formation と呼ばれるが，本菌の場合はかような形成の仕方以外に仮菌絲細胞の中央部から両側に Blastospore を着生して十字形を形成することがある（附図１）.

醗酵性から見れば *Candida albicans* (Robins) Berkhout, *C. clausenii* Lodder et van Rij に近縁であるが形態的には全く異り，むしろ *Candida reukaufii* の形態に類似するが生理学的性質は全く異る．上記の如く本菌は著しい特徴をもち他菌種とはいずれの点においても一致しないので新種と認め *Candida polymorpha* nov. spec. と命名し次の如き標式を与える．

Description of a new yeast

The synopsis of *Candida polymorpha* nov. spec.……S_5

Growth in wort: After 6 days at 25°C cells have variable shape and size: ellipsoid, oval to longish and other forms. The ellipsoid cells are about (2.4～3.3)×(3.2～5.6) μ; the long cells measure (1.6～2.4)×(11.2～16.0) μ. The various types of cells are shown in Figure 1. A little sediment and a ring are formed slightly.

Streak culture on wort agar: After 7 days at 25°C the color of the streak culture is greyish white. The streak culture is moist, waxy, raised, fine granular, a little striped at the border and slightly sinuous edges.

Giant colony on wort gelatin: see Figure 2.

Pseudomycelium formation: The development of pseudomycelium belongs to the type "Blastodendrion" and very characteristic. There is little difference between the cells of the pseudomycelium and blastospore. The cells of them are swollen in the middle or at one end. The blastospores or branched cells arise at the terminal or pleural of the pseudomycelium cells. Cross formation is often observed as shown in Figure 1.

Fermentation: Glucose only fermented weakly.

Sugar assimilation: Glucose, saccharose and maltose are assimilated.

Acid from sugars: Glucose only.

Assimilation of potassium nitrate: Absent.

Ethanol as sole source of carbon: Growth.

Growth in Litmus milk: No visible change.

Salt-tolerance: Thin pellicle on glucose broth containing 20 g NaCl/100 cc.

Optimum-temperature and-pH: Opt. temp. about 30°C and opt. pH 5.0～7.2.

The thermal death point: 60°C in 10 minutes.

Cryptococcus Kützing emend. Vuillemin

6) *Cryptococcus* spec. S_1

本菌は一般性質検査後，保存中に死滅し澱粉質生成を確認することが出来なかつたので単に所属を推定するに留める．胞子を形成せず，一般培養所見は *Bullera* 属を想起させ，Ballistospore を確めるため鏡像形成を試みたが 4 ケ月後においても形成しなかつた．仮菌絲を形成せず *Candida* 属とは思われないが Baltatu[13]が *Mycoderma decolans*

Will 等で認めた母細胞から小細胞を多数形成するのと類した現象即ち細胞から小突出物を形成し，多い場合は金米糖状を呈しそれが分離後発育するのを Bouillon あるいは Sabouraud 寒天で確認したが，この事実は *Zygosaccharomyces variabilis* でも観察されているので必ずしも *Mycoderma* の特色とは考えられず従つて *Candida* 属ではない．然し *Cryptococcus* 属の特色である Capsule 形成が麦芽汁膠の陳久培養で認められかつ非常に好気的で醗酵能を欠くことから本属に入れるのが至当であると考えられる．

Cryptococcus 属中に近縁菌を見出し得ないが前記理由により *Cryptococcus* spec. S_1 と記載するに留める．

考　　察

先に *Debaryomyces kloeckeri* を3株分離しその来源から考え興味ある事実であることを指摘したが，今回その不完全世代と見做される *Torulopsis famata* を2株分離することが出来た．*Torulopsis famata* は *Debaryomyces kloeckeri* と同様に主として動物性物料に由来し食品からは僅かに1株が冷蔵肉から分離されているにすぎない．*Candida mesenterica* は *Pseudomonilia* と称されたもので1株を得たが味噌から分離された *Pseudomonilia Miso* A, B や *P. miso* Mogi に恐らく近縁のものであろう．また *Candida parapsilosis* 3株を得たがこれは従来ほとんどすべて人体から分離されたもので Lodder et van Rij によれば27株のうち22株が人体に由来し1株が動物からで皮膚，爪等の病患部から得られていることは先の *D. kloeckeri* の場合と同様である．然し本邦では小林氏により葡萄・空中からも分離されている．又 *Cryptococcus* 属は十分明らかでないが *Torulopsis albus* (Saito) Lodder var. *miso* Mogi (*Cryptococcus albus* (Saito) Skinner の変種となる)，*Torulopsis nadaensis* Saito et Oda (*Cryptococcus diffluens* (Zach) Lodder et van Rij) が醗酵製品から得られているので本属菌の塩辛中における存在もあながち否定し去るわけには行かない．

要するに塩辛中の酵母が先に報告した *Debaryomyces* 属と共に *Candida parapsilosis* の如く従来動物性物料に由来するものが多いことは供試原料自体が筋肉・内臓の如き動物蛋白とその分解物を主とする点から考え，これら酵母がかかる基質に適合した特別の代謝機能を有しているか否かは興味ある問題と考えられ別に研究中であるが，両属酵母が広く分布することは他の醗酵食品とは異る菌相上の特色であると考えられる．

総　　括

名種塩辛類から分離した無胞子酵母8菌株の分類所属を検討し，次の如く決定した．

Torulopsis famata (Harrison) Lodder et van Rij 2株，*Candida mesenterica* (Geiger) Diddens et Lodder 1株，*Candida parapsilosis* (Ashford) Langeron et Talice 2株，同変種 *Candida parapsilosis* var. *intermedia* van Rij et Verona 1株の4既知酵母と新種 *Candida polymorpha* nov. spec. 並びに未確定の *Cryptococcus* spec. S_1.

この研究は九州大学農学部水産化学教室で行つたもので終始懇篤な御指導を賜つた恩師富安教授に謹んで深謝の意を表する．なお文部省科学研究費（富安教授）に拠つた，記して謝意を表する．

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Résumé

A taxonomic study of eight asporogenous yeasts which were isolated from the various kinds of "Shiokara" was carried out and the description of a new species was made.

The identified species by the classification of Lodder & van Rij (1952) are as follows:

Torulopsis famata (Harrison) Lodder et van Rij ····· 2 strains
Candida mesenterica (Geiger) Diddens et Lodder ····· 1 strain
Candida parapsilosis (Ashford) Langeron et Talice ····· 2 strains
Candida parapsilosis var. *intermedia* van Rij et Verona ····· 1 strain
Candida polymorpha nov. spec. ····· 1 strain

The one strain of test-yeasts was regarded as *Cryptococcus* species from some aspects of its general features.

A yeasts-flora of these foods was discussed.

水産醱酵食品の酵母に関する研究（第8報）

各種塩辛中の酵母及び細菌の来源並びに意義について

銭　谷　武　平

Yeasts occurring in fishery-fermentation products

Part 8. On the origin and the significance of yeasts and bacteria in various "Shiokara"

Buhei Zenitani

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緒　　言

醱酵食品の製造においては種々の微生物の複雑な作用によつて熟成し食品特有の香味を醸すもので，各醱酵食品についてそれぞれ詳細な微生物学的研究が行われている．農産醱酵食品においては醤油・味噌酵母のみならず細菌類についても諸氏の研究があり，またPickles の酵母，細菌に関しても多数の研究があつて，各食品の菌相は勿論有用・有害菌が確かめられ製品の保蔵・品質向上に多大の貢献をしている．しかるに水産醱酵食品は菌学的には古くから着目され酵母菌については喜多氏,[1]細菌類については町田氏[2]により最初に研究されたにもかかわらず，その後は前記他種製品のように詳細には研究されていない．

著者はこの食品類が栄養，食品貯蔵または廃棄物利用等の見地から微生物学的にも興味ある製品であるから本研究に着手し各種の塩辛類の微生物全般について検討した．酵母菌に関しては逐次報告してきたが[3]本報では更に細菌類の研究[1]を併せて水産醱酵食品の製造に関与する微生物の種類，分布について総括し，これら微生物の来源及び製造における意義について考察する．

I. 塩辛中の細菌並びに酵母の菌数

Table 1. Population of bacteria and yeasts in some kinds of "Shiokara."

Products	NaCl %	pH	Bacteria per gram	Yeasts per gram	Note
Ika	17.4	5.2	4,470,000	1,600	Brined liquid was used as samples.
	15.2	5.4	3,580,000	1,850,000	
	7.9	5.6	24,300,000	95,000	
Katsuo	21.7	5.0	1,550	500	All solid matter was used as samples.
	15.2	5.5	230,000	25,000	
	14.8	5.6	1,800	150	

Note: Counts of both microbes were made after 5 days at 30°C conventionally by the plating methods with wort agar (10% NaCl, pH 5.0) in the case of yeasts and by nutrient broth agar (10% NaCl, pH 7.5) in the case of bacteria.

塩辛類は下等水産動物，ウニ，アサリ，イカ等を原料にするものと魚類，カツオ，ブリ等の内臓を利用するものとがあつてその種類が多い．従つて品質は原料の鮮度，食塩濃度，熟成日数等によつて相違する．しかし各製品中の微生物作用を確めるには細菌及び酵母の菌数を知る必要があり数個の試料についてその概数を便宜的に麦芽汁寒天と肉汁寒天とを用いて測定して見た．細菌類は酵母菌よりも多く Table 1 のように供試品によつて著しく相違した．また酵母菌が細菌と同様に 10^6 程度も存在する製品のあることから有用酵母の接種も考慮される．

II. 塩辛から分離した酵母と細菌

A) 酵母菌:— 従来塩辛類をはじめ各種の水産製品中の酵母はすべて *Torula* 属であると報ぜられていたが[1,5]著者は胞子形成法を検討して大多数が真正酵母であること及び不完全菌の仮菌絲形成法を検討して概ね *Candida* 属であることを明らかにした．

Table 2. The identified species of yeasts and bacteria occurring in various kinds of "Shiokara".

Yeasts	Bacteria
Saccharomyces rouxii Boutroux.	*Vibrio marinopraescens* ZoBell et Upham var.
Saccharomyces kyushuensis n. sp.	*Vibrio adaptatus* ZoBell et Upham.
Hansenula anomala (Hansen) H. et P. Sydow.	*Vibrio aquatilis* Gunther var.
Debaryomyces hansenii (Zopf) Lodder et van Rij.	*Micrococcus aurantiacus* (Schroeter) Cohn.
Debaryomyces nicotianae Giovannozzi.	*Micrococcus citreus* Migula.
Debaryomyces nicotianae var.	*Micrococcus flavus* Trevisan.
Salusus n. var.	*Micrococcus epidermidis* (Winslow and Winslow) Hucker.
Debaryomyces kloeckeri Guilliermond et Péju.	*Micrococcus conglomeratus* Migula var.
Debaryomyces oviformis n. sp.	*Micrococcus varians* Migula.
Torulopsis famata (Harrison) Lodder et van Rij.	*Achromobacter* sp.
Candida mesenterica (Geiger) Diddens et Lodder.	*Flavobacterium marinum* Harrison var.
Candida parapsilosis (Ashford) Langeron et Talice.	*Bacillus subtilis* Cohn emend. Prazmowski.
Candida parapsilosis var. *intermedia* van Rij et Verona.	*Bacillus pumilus* Gottheil.
* *Candida polymorpha* var. *shiokara* n. var.	*Bacillus repens* Gibson.
Cryptococcus sp.	*Bacillus circulans* Jordan.
	Bacillus brevis Migula emend. Ford.
	Bacillus laterosporus Laubach.

Table 2 のように *Debaryomyces* 属酵母が普遍的で次に *Saccharomyces* 属，*Candida* 属及び *Torulopsis* 属の順序になつた．*Saccharomyces rouxii* は醤油諸味，辛味噌，溜諸味及び蜂蜜から分離されているが主に雲丹塩辛に見出された．頻繁に分離した種類は *Debaryomyces nicotianae*, *D. kloeckeri*（不完全期は *Torulopsis famata*）

及び *Candida parapsilosis* の4種とその変種であった．また *Debaryomyces oviformis* nov. spec. は麦芽糖から生酸しなかったが最近醤諸味から麦芽糖を同化しない *D. tamari* Ohara et Nonomura[6] が分離されたのでこのような系統の酵母は含塩性醗酵製品に分布していると思われる．

B）細菌類：― 従来細菌の分類学的研究がなされた製品は主に北海道産試料かまたは塩汁，魚醤油のような特殊な製品である．魚類内臓を原料としたものについては研究されていないので著者は九州産の各試料（カツオ，サバ，シイラ，イカ，ウニ等）の細菌[4]について研究し Table 2 のように 19 種に整理した．

Vibrio marinopraesens, V. adaptatus, Flavobacterium marinum のような海洋細菌の変種を分離した．これらがいずれも好塩性であることと乳酸菌系統のものが分離されないこととは農産製品と違う点である．

他の種類は種々の食品，魚体，用塩などに見出されるもので特に *Micrococcus aurantiacus, M. varians* 及び *Bacillus subtilis* が屢々分離された．

上記微生物の種類から見て酵母では *Debaryomyces* 属と *Candida* 属が広く分布し *Saccharomyces* 属は所謂 *Zygosaccharomyces* 属に限られ，また細菌類では普通食品類にくらべて好塩性の海洋細菌 *Vibrio* 属などの存在することが特異な点と思われる．

III. 各製品に見出された酵母と細菌

各製品の微生物は風味構成の重要因子であつて個性的風格を生ずる原因であることは茂木氏[8]が既に味噌について指摘されている．塩辛類は味噌・醤油類にくらべて性状・品質がはるかに雑多であるが供試製品を Table 3 のように区別し微生物の製品別の分布を示した．

水産醗酵食品の製造上に有害な菌は一応この研究から指摘し得るが有用菌の識別はなお将来の研究にまたねばならない．*Saccharamyces rouxii* と *S. kyushuensis* nov. spec. は脱水度の低い泥雲丹に往々異常醗酵を起させ製品を軟化せしめて香味を損なう．富安・著者[9]はその原体が前記2種であることを明らかにし，本質的には醤油・味噌の湧き酵母と全く同種類かまたは極めて近縁なものと考えた．しかしイカ塩辛の異常醗酵は *Bacteroides halophilus* Hanzawa et Sasaki[10] に起因する由である．

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* **訂正**　前報[7]投稿後に *Candida polymorpha* nov. spec. と同名称の新酵母が発表された（小原・野々村：農化，**28**, 717, 1954）．この菌と著者が分離命名した *Candida polymorpha* とは共に多形態で葡萄糖のみを発酵し重要な性質は全く一致する．ただしガラクトース及び酒精同化性は相反し Cross formation が著者の菌には認められるが両氏の酵母には見られない．また三角形細胞は著者の場合にはむしろ円錐型に近い点において相違する．よつて新変種と認め *Candida polymorpha* Ohara et Nonomura var. *shiokara* nov. var. と訂正する．

Note: *Candida polymorpha* nov. spec. reported in the previous paper is emended as *Candida polymorpha* Ohara et Nonomura var. *shiokara* nov. var. due to the publication of the similar yeast by Ohara & Nonomura (J. Agr. Chem. Soc. Japan, **28**, 717, 1954).

Table 3. The distribution of yeasts and bacteria in various kinds of products.

Products	Yeasts	Bacteria
Uni-shiokara.	*Saccharomyces rouxii.* *S. kyusuensis.* *Debaryomyces nicotianae.* *D. nicotianae var. salsus.* *D. kloeckeri.* *D. hansenii.* *Candida parapsilosis.* *Hansenula anomala.*	*Bacillus subtilis.* *Micrococcus citreus.*
Ika-shiokara.	*Saccharomyces rouxii.* *Debaryomyces nicotianae.* *D. hansenii.* *Candida mesenterica.*	*Micrococcus varians.* *M. conglomeratua.* var. *M. epidermidis.* *Vibrio marinoparaescens* var. *V. adaptatus.* *Bacillus repens.* *B. subtilis.*
Katsuo-shiokara	*Debaryomyces nicotianae.* *D. kloeckeri.* *D. oviformis.* *Torulopsis famata.* *Candida polymorpha* var. *shiokara.*	*Micrococcus citreus.* *M. epidermidis.* *M. flavus.* *M. freudenreichii.* *Vibrio adaptatus.* *V. aquatilis* var. *Bacillus subtilis.*
Miscellaneous products	*Saccharomyces rouxii.* *Torulopsis famata* *Candida parapsilosis.* *C. parapsilosis* var. *intermedia.*	*Micrococcus varians.* *M. citreus.* *Bacillus brevis.* *B. circulans.* *B. laterosporus.* *Flavobacterium marinum* var.

細菌の接種試験によると *Vibrio* 属及び *Achromobacter* 属の好塩細菌が腐敗を促進し *Bacillus* 属は塩辛中では植物性食品の場合とは異り作用が少いので随伴菌と考えられる.

IV. 塩辛中の微生物の来源について

有害微生物の来源を知ることは製造上重要なことで特に水産醱酵食品, 就中塩辛類は製造工程において全く加熱処理を受けないから原料と共に搬入される微生物の影響をうけることは当然である. 海洋中の微生物が原料動物体に附随する機会の多いことから普通の食品製造に関係ある微生物とは来源が違うものであると考えねばならぬ.

A) 酵母菌:— ZoBell[11]は海洋酵母として *Endomyces*, *Saccharomyces*, *Torula*,

Mycoderma, *Dematium* 及び *Oidium* 等の諸屬を擧げて陸地からの分離とは無關係に存在するものの如く，外洋ではむしろ細菌よりも多いこともあると記している．本邦では海洋酵母の研究は殆どなかつたが最近富安・木原両氏[12]が海藻，海泥から *Saccharomyces*, *Hansenula*, *Geotrichum*, *Candida*, *Trichosporon* など多數の種類を分離していることから海洋から由來することも當然考えられる．特にアサリ塩辛にはアサリの食性から見て海洋在來種が存在する可能性が大である．Wehmer[5]はタラ塩汁中の食塩酵母は海水に由來すると考え，木村・小谷両氏は雲丹異常醱酵の原体である *Torula* がウニ卵巣中に見出されないことから空中から來ると考えた．また Mrak & Phaff[13]は野菜塩汁中の *Debaryomyces* は Air born yeast であるとした．最近 Skinner & Connel[11]は非醱酵性・色素不生産性酵母は空中よりも人体皮膚面が有力な棲息所と発表していることから考えると取扱者の清潔さにも注意すべきである．

B）細菌類：— 既述のように海洋細菌の存在は明らかに原料動物体と共に搬入されたものであつて，食塩からの混入も長尾氏[15]等の研究で明らかである．

要するに水産醱酵食品の場合は一般食品製造と同様に原料，用塩，用水，容器や外界から由來するが特に原料に附随してくる海洋性細菌と酵母が悪影響をもたらす懸念があるから洗滌操作などにも深い関心をよせるべきである．

V. 熟成における微生物の意義

水産醱酵食品はその種類が多く熟成状態もそれぞれ相違する．奥田氏[16]は塩辛熟成は原料の酵素，微生物及び食塩の協同作用によると考えたが熟成現象に対する見解は研究者により必ずしも一致していない．呈味の主体であるアミノ酸類の増加という点から見ると自己消化酵素，特にイカ塩辛においては肝臓酵素の作用が最も強力であつて，塩辛製造もアミノ酸の増加のみに主眼をおくとほとんど原料中の酵素作用に帰せられるが，所謂滋味の構成・風味の醇化を考慮すると後熟期における細菌並びに酵母菌の作用が極めて重要な意義をもつ，清水氏[17]はカツヲ塩辛の熟成において細菌類がモノアミノ酸類を分解しその作用をむしろ好ましからぬものと考え，他方イカ塩辛で関根・松井両氏[18]は後熟期における細菌・酵母の作用を挙げ，また長尾氏は細菌数の変化から後熟期によく作用することを指摘された．

これらの微生物は前熟期に生成したアミノ酸，有機塩基等に作用し低級脂肪酸やその中間体等を生成しあるいは生成物の間に縮合を起さしめるなどして特有の香味の調和が行われるものと思われる．又微生物の代謝作用のみならず菌体細胞の自己消化作用に基く酵素作用や細胞内成分の溶出等も看過することが出来ない．酵母菌は一般農産食品例えば醤油製造における如き醱酵作用に基く芳香附与よりはむしろ醱化期の *Debaryomyces* 属や *Candida* 属が普遍的でその作用を重視すべきであるがこれら酵母の食品製造における意義については Césari, Césari & Guilliermond[19]が腸詰製造において *Debaryomyces* 属酵母が香気改良と腐敗防止に役立つことを指摘しているにすぎない．これら菌類の生理作用については別に研究中で追つて報告の予定である．熟成中における細菌並びに酵母の作用は相錯綜し複雑であるが恐らく両者が協同し塩辛特有の風味の醇化に役立つものと思われる．

要 約

1. 従来の研究では水産加工品から分離された酵母菌はすべて *Torula* 属と分類されていたが胞子生産法を検討した結果供試37菌株中29菌株について胞子生産を確めた.

2. 真正酵母は *Debaryomyces* 属19株, *Saccharomyces* 属9株及び *Hansenula* 属1株で前2属が主体をなすことは他の醱酵食品と対比して著しい特徴である. また無胞子酵母は *Candida* 属5株で *Torulopsis* 属2株は *Debaryomyces* 属の不完全期と認められた. なお未確定菌株として *Cryptococcus* 属1株を得た.

3. 塩辛中の *Saccharomyces* 属は味噌酵母の特性に共通し旧分類の *Zygosaccharomyces soja, Z. japonicus, Z. major* var. *miso* 及び *Z. miso γ* の如く醤油・味噌酵母を包含したがすべて *Saccharomyces rouxii* に統合した.

4. *Debaryomyces* 属は最も普遍的に見出されその殆ど大部分は著者の分類法[20]の第II群, 即ち葡萄糖, 蔗糖及び麦芽糖から生酸する種類で *Debaryomyces nicotianae* 8株, *D. kloeckeri* 3株と第I群即ち葡萄糖, 蔗糖, 麦芽糖及び乳糖から生酸する *D. hansenii* 2株が確められた.

5. 無胞子酵母菌はすべて非乃至弱醱酵性であつて *Candida mesenterica, C. parapsilosis* 及びその変種 *intermedia, C. polymorpha* var. *shiokara* の計4株で *Torulopsis famata* は *Debaryomyces kloeckeri* の不完全期に該当した.

6. 塩辛中の細菌は耐・好塩性をもち *Micrococcus* 属7種, *Bacillus* 属6種, *Vibrio* 属3種と *Achromobacter* 属並びに *Flavobacterium* 属各1種を得, 特に好塩性の海洋細菌の存在は注目すべきものである. 熟成においては *Micrococcus* 属が最も主要な作用をなし *Bacillus* 属はその作用が著しくないのでむしろ随伴細菌と見るべきである.

7. 製造上から有害菌と認むべきものは *Saccharomyces rouxii* 及び *S. kyushuensis* の2種で雲丹塩辛の異常醱酵の原体となり製品を軟化し香味を損なう. また好塩性海洋細菌である *Vibrio* 及び *Achromobacter* 属菌の過度の作用は腐敗と極めて密接な関係がある.

8. 塩辛中の酵母並びに細菌類は一般醱酵食品のそれとは異り原料動物の棲息環境である海水あるいは海泥等から相当搬入されるものと考えられるので特に洗滌操作に深い考慮を払うべきである.

9. 製品の熟成作用の解明はなお今後の研究に俟たねばならないが前熟期に生成したアミノ酸, 低級脂肪酸等を微生物が分解し菌体自身の酵素作用と相俟つて滋味・香気の調和に微妙に作用し製品特有の香味を醸成すると思われる.

10. この研究において *Saccharomyces kyushuensis* nov. spec., *Debaryomyces oviformis* nov. spec., *D. nicotianae* var. *salusus* nov. var. 及び *Candida polymorpha* var. *shiokara* nov. var. の新酵母4種を分離した.

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Résumé

In the present paper, the identified species of yeasts and bacteria, and their distribution in each product were summarized. The significance of these microbes, together with their origin, in the processing of the products, was discussed.

It seems that the bacteria, associating with the yeasts, are mainly concerned in the ripening process, especially *Micrococcus* and *Debaryomyces* or *Candida* having oxidative abilities are responsible for this process. On the other hand halophilic *Vibrio* or *Achromobacter* which presumably originated from marine, and *Saccharomyces* seem to be unfavorable for the manufacture of this product.

カツオ及びウニ塩辛中抗菌性物質の存在について

(抗菌性油とそれに対する阻害作用)

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(Antibiotic oil and reversal action to it)

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緒　　言

塩辛は魚介類の筋肉，内臓等を塩漬にして熟成させたもので，その防腐作用は専ら食塩に帰せられているが塩辛が腐敗することも屢々指摘されている．塩辛の食塩含量と腐敗との関係についてイカ塩辛で島田，馬場[1]は 20°C に於て 10% では 13 日目に，20% では 17 日目に悪臭を発し食塩を 30% 位迄添加しなければ腐敗を防止し得ないと述べ，カツオ塩辛について清水[2]はアンモニア発生状態を研究し食塩含量 20% ではアンモニアの発生が少く，15% 及び 10% では 3 ヶ月後には相当増加することを認め食塩の必要量は 15% 以上であると結論した．ウニ塩辛は特に異常醱酵し易く，木村，小谷[3,4]はその原因として 2 種の *Torula* 属酵母を分離し，防腐方法としては 22°C 以下に貯蔵すること及び水分含量を 50% 以下にすることを勧めたが，現在では長期間の保蔵には防腐剤としてアルコールを添加している．又最近富安，銭谷[5]はウニ塩辛の異常醱酵は *Saccharomyces rouxii* Boutroux 及び *Saccharomyces kyushuensis* Zenitani に基因し，ビタミン K_3 の添加によつて防止し得ると述べている．

上記の如く塩辛のうちではウニ塩辛が最も変敗し易いが，カツオ塩辛は比較的腐敗し難く，製造時期等の関係もあるが夏でも市場で見受けられる．又戦時中魚介類煮汁より代用調味料の製造が試みられたが腐敗し易く相当量の食塩を添加しても防腐し得なかつたし，アサリ塩辛も比較的腐敗し易いものである．斯くの如く原料魚介類の種類によつて貯蔵性に差異の生ずることより考えると塩辛の防腐作用を食塩のみに帰することは妥当でなく他の因子を考慮に入れなければならない．

塩辛は魚介類の筋肉，内臓及び卵巣等を原料としているから微生物の発育促進物質が含有されていることは当然想像されるし，また一方山田，篠山[6]は塩蔵カツオ肝臓中に防黴成分が含まれていることを報告している．

塩辛中の油は不飽和度の高い脂肪酸を含有しており，深山，猿谷[7]は 20% 食塩水に塩蔵したサバの脂肪について，谷川等[8]は魚味噌熟成中の エーテル可溶物についてそれぞれ酸価の増加及び沃素価の減少を認め，また Banks[9]は 食塩が魚肉中に浸透すると 油の酸敗が促進されると述べている．これ等のことから塩辛中の油は 相当酸化されているものと考えられる．先に著者等[10]は魚油の酸化によつて 抗菌作用の出現することについて報告したが，塩辛中の酸化油が抗菌作用を有し，塩辛の防腐作用に関係があるのではないかとの考えのもとに防腐力の強いカツオ塩辛と防腐力の弱いウニ塩辛との防腐効果の 差異の原因について検討を試みた．その結果抗菌性酸化イカ肝油を魚肉に添加すると 比較的低濃度の食塩でも魚肉は防腐されることを明かにした．またカツオ及びウニ塩辛中のエーテル可溶物は抗菌作用を示し，カツオ塩辛中にはこのエーテル可溶物の 抗菌作用に対する阻害物質は含有されていないが，ウニ塩辛中には強力な阻害物質が含有されていた．仍てウニ塩辛が腐敗し易いのはこの阻害物質のためにエーテル 可溶性の酸化油が全く抗菌作用を示さないことに一原因があると考えた．

実 験 の 部

I. 抗菌性酸化魚油及び食塩の防腐効果

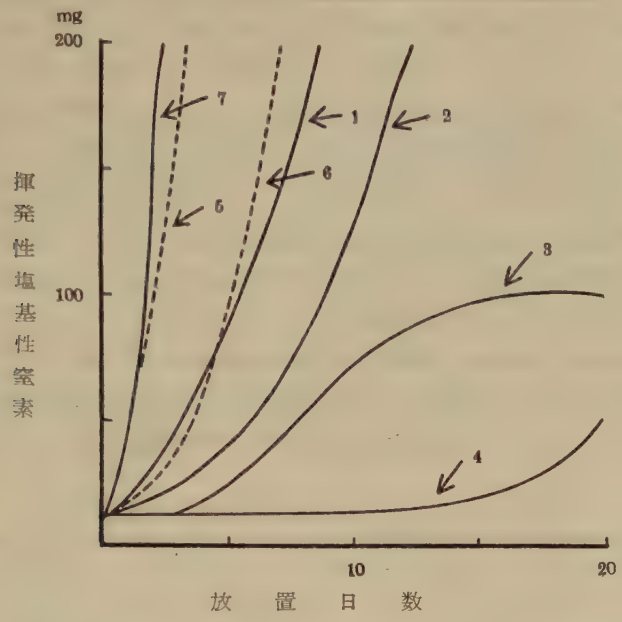
塩辛中に於て酸化油が防腐効果を有するか否かを検討するには含油量の 可及的少い魚肉に食塩及び酸化油を添加してその防腐効果を吟味するのが最良の方法であると考えられる．仍て含油量の少い魚肉としては 磨砕した カレイ の筋肉を，酸化魚油としては 著者等の方法[10]即ち精製したイカ肝油（沃素価 185.9，過酸化物価 0.8）に $\frac{M}{100}$ になる如く Cu-stearate を添加して後，酸素吹込み下で 100°C に於て 1.5 時間酸化する方法により得た抗菌性酸化イカ肝油（抗菌力 1,000）をそれぞれ試料として用いた．

食塩及び酸化イカ肝油の防腐効果は第1図に示す如くであつた．Labried and Gibbons[11]はタラ肉の圧搾汁に種々の濃度の食塩を添加し 21°C に於て 20% 以下では Trimethyl-amine 及び揮発性窒素が増加すると述べているが，著者等の結果では 30° C に於ては 20% の食塩濃度でもカレイ筋肉は完全には防腐されなかつた．一方酸化 イカ 肝油のカレイ筋肉に対する防腐効果は極めて微弱で 20% 添加しても殆ど効果は認められなかつた．

塩辛の食塩含量は 15～20% であるが 第1図で 明かな如く 食塩を 15% 添加すると揮発性窒素の増加は緩慢であるので，食塩含量 12.5% に於て酸化イカ肝油を 5% 及び 10% 添加して食塩と酸化イカ肝油の協同作用による防腐効果を試験した．その結果は第2図に示した如く防腐効果が顕著に表われた．カツオ塩辛の含油量は 3～10% であり食塩含量は 15～20% であるので，酸化油が塩辛の防腐に対して食塩との協同作用により相当顕著な効果を示し得る可能性を知つた．

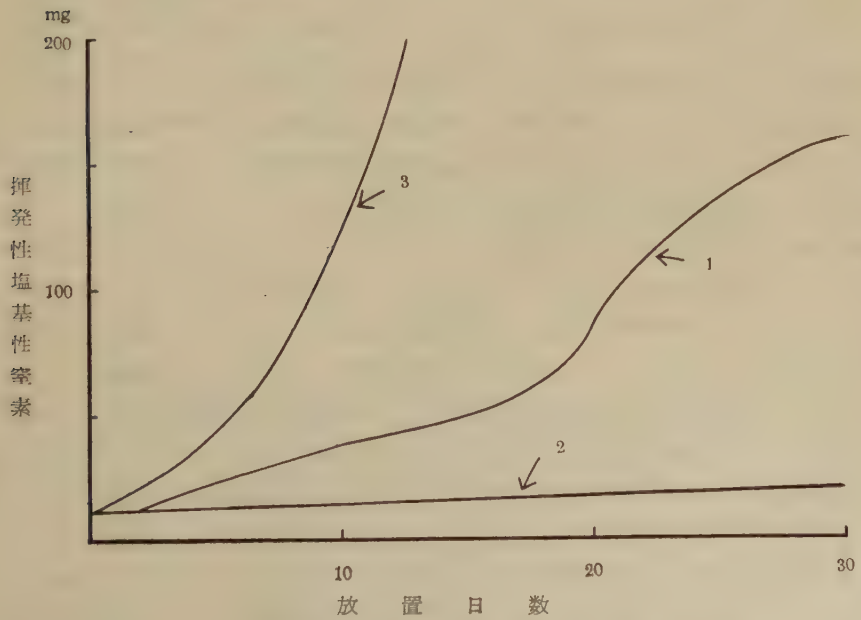
II. 塩辛中の酸化油の抗菌作用並びに塩辛中それに対する阻害物質の存在

前述の如く酸化魚油は食塩との協同作用により強力な防腐効果を表わすので塩辛中の油状物質を抽出し，その収量及び抗菌力を測定した．即ち市販のカツオ塩辛（食塩含量 14.0%，揮発性塩基性窒素 89.3 mg）及び ウニ塩辛 （食塩含量 17.9%，揮発性塩基性窒素



第 1 図. カレイ筋肉の防腐に対する食塩及び酸化イカ肝油の効果 (30 °C).

1．食塩 10％　2．食塩 12.5％　3．食塩 15％　4．食塩 20％
5．酸化イカ肝油 10％　6．酸化イカ肝油 20％　7．対照（食塩・酸化イカ肝油無添加）



第 2 図. カレイ筋肉の防腐に対する食塩及び酸化イカ肝油の協同効果 (30 °C).

1．食塩 12.5％＋酸化イカ肝油 5％　2．食塩 12.5％＋酸化イカ肝油 10％
3．対照（食塩 12.5％）

97.5 mg）を試料とし，等量の脱水芒硝を加えて充分混和した後共栓三角フラスコ中で試料に対し2倍量のエーテルを用いて室温で3回抽出を行つた後，2回目の溶剤としてはHardwick等[12]が *Bacillus mycoides* に対するペプトン中の発育阻止物質であるペプタイドと結合状態にある油状物質の抽出に用いて好結果を得た醋酸エチールを用いてエーテルの場合と同様に抽出を行つた．各抽出物はクライス反応陽性で明かに酸化された状態にあり，それ等の収量，抗菌力及び性状を第1表に示した．即ちカツオ塩辛中の抗菌性物質はエーテル可溶物及び醋酸エチール可溶物の両者に存在し，それ等の収量は1.5，0.4%であり抗菌力はそれぞれ1,333及び1,000であつたが，ウニ塩辛ではエーテル可溶物にのみ抗菌性物質が存在し，収量は2.8%で抗菌力は200であつてカツオ塩辛と比べて抗菌力が極めて小であつた．然し乍ら腐敗し易いウニ塩辛中にも抗菌性物質が存在しているのでこの結果のみからではウニ塩辛の腐敗し易い原因を十分に説明することは出来ない．

第1表．　カツオ及びウニ塩辛中の抗菌性油状物質の性状．

塩辛の種類	溶剤	収量(%)	クライス反応	抗菌力	沃素価	過酸化物価
カツオ	エーテル	1.5	＋	1,333	119.5	26.4
	醋酸エチール	0.4	＋	1,000	146.9	12.3
ウニ	エーテル	2.8	＋	200	127.5	15.0
	醋酸エチール	4.3	＋	0	157.6	10.3

第1表に於てウニ塩辛の醋酸エチール可溶物のみが抗菌作用を全く示さなかつたので，その原因を明かにするために抗菌性酸化イカ肝油にこの醋酸エチール可溶物を添加して抗菌力を試験したところ，酸化イカ肝油の抗菌作用が阻害されたのでウニ塩辛の醋酸エチール可溶物中には酸化油の抗菌作用を阻害する物質が存在していることを知つた．仍て塩辛には酸化油の抗菌作用に対する阻害物質が含有されている懸念があるのでその有無について追求した．その結果カツオ塩辛には阻害物質は全く含有されていないが，ウニ塩辛には明かに存在し，その阻害物質はアルコール可溶部に存在し，しかもウニ塩辛のエーテル可溶物の抗菌作用はこのアルコール可溶物の等量の添加によつて全く阻止されてしまうことを知つた．ウニ塩辛中の抗菌性物質であるエーテル可溶物と抗菌性物質に対する阻害物質であるアルコール可溶物の含量はそれぞれ2.8%，20.7%であるのでウニ塩辛では抗菌性エーテル可溶物はその抗菌作用を全く阻止され，ウニ塩辛の防腐作用は食塩のみによつていることになりその結果腐敗し易いのである．然るにカツオ塩辛では酸化油の抗菌力は強く且つこの抗菌作用に対する阻害物質は全く含有されていない．しかもカツオ塩辛には3～10%のエーテル可溶物が含有され，食塩含量は15～20%であるからその防腐作用は抗菌性油と食塩の協同作用による比較的強力なものであり従つて腐敗し難いのであると説明し得ると思う．

摘　　要

1. カツオ塩辛とウニ塩辛の防腐効果が異る原因について検討を試みた.

2. 抗菌性酸化イカ肝油の魚肉に対する防腐効果は極めて弱いが食塩と併用すると協同作用によりその効果は大きくなる.

3. カツオ塩辛及びウニ塩辛中には共に抗菌性酸化油状物質が含有されている. 後者にはこの抗菌性酸化油状物質に対する阻害物質が含有されているが, 前者には含有されていない.

4. カツオ塩辛が腐敗し難いのは抗菌性酸化油状物質と食塩との協同作用により防腐され, ウニ塩辛が異常醱酵し易いのは阻害物質のために抗菌性酸化油状物質の抗菌作用が全く阻止され食塩のみにより防腐されていることに基因する.

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Résumé

It has been recognized that the preservation of "Shiokara" is attributed to the action of sodium chloride. "Katsuo-shiokara" is scarcely spoiled, but "Uni-shiokara" causes frequently gaseous fermentation. This difference in the preservation of two kinds of "Shiokara" can not be explained solely by the action of sodium chloride, so we considered that there must exist some unknown antiseptic substances in "Katsuo-shiokara". According to this assumption, investigations were carried out by the authors and the following results were obtained.

1) The antibiotic oxidized calamary oil does not preserve white muscle

of fish, but it is effective when it co-operates with a certain amount of sodium chloride (Figs. 1, 2).

2) The antibiotic oxidized oils are contained in both "Uni- and Katsuo-shiokara" (Table 1).

3) "Uni-shiokara" contains a reversal substance for the antibiotic oxidized oil, so the antibiotic oxidized oil does not exhibit the antiseptic effect at all, whereas "Katsuo-shiokara" does not contain a reversal substance, so it is preserved by antibiotic oxidized oil and sodium chloride.

魚体成分の抗菌作用に関する研究—Ⅶ. 酸化イカ肝油の抗菌作用について

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Studies on the Antibiotic Action of Fish Components—Ⅶ. On the Antibiotic Action of the Autoxidized Calamary Oil

Yukio Tomiyasu and Masamichi Toyomizu

In the present work the spectrum and the mode of action of the antibiotic autoxidized calamary oil and the reversal of inhibitory action of it were investigated, and the following results were obtained.

1) The autoxidized calamary oil inhibits the growth of bacteria independently of their Gramstaining characters, and it shows weak antibiotic activity on molds and some strains of yeasts and Fungi imperfecti.

2) The antibiotic action of the autoxidized calamary oil causes the prolongation of the lag period and is effective when added at the beginning of culture, while the autoxidized calamary oil stimulates the growth of organisms in certain cases when added at the stationary period.

3) The antibiotic action of the autoxidized calamary oil is not reversed by sulfhydryl compouds and surface active substances such as cholesterol, lecithine and α-tocopherol; but it is reversed by unsaponifiable matter of plant oil, medicinal yeast and among tested adsorbents only by activated charcoal.

4) It seemed that the antibiotic action of the autoxidized calamasy oil depended on an oxidative product, whose mode of action differed from those of unsaturated fatty acids, saturated fatty acids and surface active substances.

著者等は前報[1]に於てイカ肝油が酸化により抗菌作用を出現することを明かにした。直鎖脂肪酸及びそれ等の石鹸の抗菌作用については古く Bayliss[2] の研究があり，その後数多くの研究が行われ，最近Kodicek [3] は不飽和脂肪酸の抗菌作用について界面活性剤としての見地からその作用機構を追求した。酸化油及び酸化不飽和脂肪酸の抗菌作用に関する研究は極めて少いので本報に於ては酸化イカ肝油の抗菌性を明かにする目的で抗菌スペクトラム，抗菌型式及び抗菌作用に対する阻害物質について研究を行った。

実　験　の　部

Ⅰ．酸化イカ肝油の抗菌スペクトラム

供試酸化イカ肝油は M/100 になる如く Cu-stearate を添加した精製イカ肝油（沃素価 185.9，過酸化物価 0.8）を乾燥酸素吹込み下に 100°C，1.5時間酸化したものである。抗菌力の検定は Agar streak method により次の如くして行った。即ち18株の細菌類は Bouillon 寒天培地で 37°C に於て 24 時間，26 株の真菌類は Peptone-Hayduck 寒天培地又は麦芽汁寒天培地で 30°C に於て 48 時間微生物の発育を完全に阻止するのに要する酸化イカ肝油の必要量を求めた。また著者等は微量の硫酸銅を含んだ培地に魚油を添加すると抗菌作用が出現し，この現象は魚油の酸化に基くと指摘したが[4]，培地に硫酸銅を 5×10^{-4}M になる如く添加した場合に微生物の発育を完全に阻止するのに要する新鮮イカ肝油の必要量をも測定した。それ等の

1955 年 2 月 7 日受理

結果の一部を Table 1. に示した。不飽和脂肪酸の抗菌作用が Gram 染色性と関係があり，Gram 陽性菌に抗菌性を有することを KODICEK[3] 及び NIEMAN[5] が指摘しているが，著者等の結果では酸化イカ肝油の抗菌作用は細菌類に対しては Gram 染色性には殆ど無関係に作用した。真正酵母菌及び不完全菌に対しては抗菌作用の弱い場合もあり，また抗黴性は一般に弱かつたが *Penicillium* 属の2菌株に対しては *Aspergillus* 属の7菌株に対してよりも遙に強く作用した。各菌株に対する酸化イカ肝油の必要量は培地に微量の硫酸銅を添加した場合の新鮮イカ肝油の必要量より小であるが両者は略々比例し，酸化イカ肝油の抗菌作用機構と硫酸銅の添加により出現する新鮮イカ肝油の抗菌作用機構とは密接な関係があることを示している。

抗菌性物質の抗菌力が培地の組成に影響されることは屢々指摘されているので *Debaryomyces membranaefaciens*（以下 *D.m.* と略記する）を供試菌として培地組成の影響について試験した結果，麦芽汁寒天培地でも Peptone-Hayduck 寒天培地でもまた Peptone-Hayduck 培地では Peptone が 0.1～1% に於ては Peptone 含量の多少に拘らず抗菌力に差異は表われなかつた。

Table 1. Antimicrobial spectrum of the autoxidized calamary oil and fresh calamary oil in the presence of $CuSO_4$ (5×10^{-4}M). (10cc Medium)

	Autoxidized calamary oil	Fresh calamary oil	Medium
Vibrio aquatilis	50 mg	75 mg	B
Pseudomonas aeruginosa	100	100	B
Micrococcus perflavus	50	75	B
〃 *subflavus*	10	25	B
Streptococcus liquefaciens	75	100<	B
Proteus vulgaris	25	100	B
Flavobacterium marinum var.	50	50	B
Bacillus subtilis	50	75	B
〃 *repens var.*	5	10	B
Escherichia coli	50	25	B
Saccharomyces sake	100<	100<	W
Zygosaccharomyces soja	50	100	W
Debaryomyces membranaefaciens	10	10	P
Hansenula javanica	100<	100<	P
Schizosaccharomyces pombe	25	75	W
Torula utilis	100	100<	P
Rhodotorula sp.	5	25	P
Oospora lactis	100<	100<	P
Mucor christianes	75	100<	P
Rhizopus japonicus	100<	100<	P
Aspergillus oryzae	100<	100<	P
Penicillium chrysogenum	25	50	P

Medium : — B : Bouillon agar, W : Wort agar, P : Peptone-Hayduck agar.

Ⅱ. 酸化イカ肝油の抗菌型式

酸化イカ肝油の微生物発育阻害現象を観察するために供試菌として *D.m.* 及び *Escherichia coli*（以下 *E. coli* と略記する）を用い，酸化イカ肝油を菌の接種前，対数期及び定常期に添加した場合の菌の発育状態を光電光度計で追求した。その結果を Fig. 1,2 に示した。BAUERNFEIND 等[6]，STRONG 等[7]，KODICER 等[8]及び THOMA 等[9] は不飽和脂肪酸の抗菌作用は主として誘導期の延長によるものであり静菌的に作用すると述べているが，著者等の実験結果でも接種前に添加した場合には *D.m.* に対しても *E. coli* に対しても同様に主として誘導期阻害が表われその結果誘導期の延長が認められたが，その添加量によつては対数期阻害は弱いこともあつた。また溶菌作用は全く認められなかつた。対数期に添加した場合には発育阻害は弱く，定常期に添加した場合にはその添加量によつては明かに発育促進現象が表われ，Linoleic acid で BAUERNFEIND 等[6]，及び STRONG 等[7] が，Oleic acid で HUTCHING 等[10]，WILLIAMS 等[11] 及び DUBOS[12] が認めた所謂 "Double action" が酸化イカ肝油でも表われた。即ち酸化イカ肝油の抗菌作用は主として誘導期の延長によるものであり菌の発育初期に添加した場合に有効であることを明かにした。

Ⅲ 酸化イカ肝油の抗菌作用に対する阻害物質

抗菌性物質の作用機構を明かにする目的で抗菌性物質に対する阻害剤の研究は数多く行われている。界面

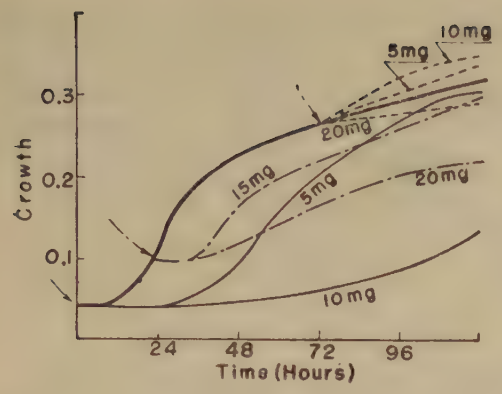

Fig. 1. Antimicrobial effect of the autoxidized calamary oil on *Debaryomyces membranaefaciens*

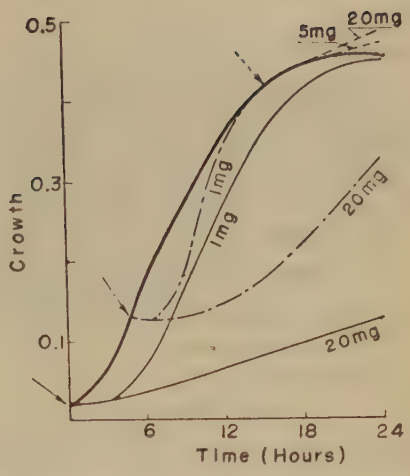

Fig. 2. Antibacterial effect of the antoxidized calamary oil on *Escherichia coli*

活性剤の抗菌作用は Cholesterol, Lecithine により阻害され，不飽和脂肪酸の抗菌作用は Cholesterol, Lecithine[3)8)14)], α-Tocopherol[3)8)] 及び或る種の蛋白質例えば血清等[3)12)]により阻害され，澱粉[15)16)17)]及び活性炭[3)8)17)]等が吸着剤として作用することにより油状物質の抗菌作用が阻害され，さらに各種抗菌性物質の作用が Cysteine 及び Glutathion の如き Sulfhydryl 化合物によって阻害されることは既に報告されている。仍て上記阻害物質について 10cc の Peptone-Hayduck 培地に酸化イカ肝油を *D.m.* の発育阻止に必要な最低量約 10 mg 添加して阻害作用の有無を試験した。その結果を Table 2. に示した。即ち酸化イカ肝油の抗菌作用は Cholesterol, Lecithine 及び α-Tocopherol の如き界面活性剤によっても，Sulfhydryl 化合物によっても全く阻害されず，吸着剤としては活性炭によってのみ阻害された。また Biotin 及び Biotin の作用をなす Stearic acid, Oleic acid によっても阻害されなかつたが椿油，胡麻油及び亜麻仁油等の植物油により阻害され，その有効物質は植物の固形油及び液体油には存しないで不鹸化部に存在した。尚培地に微量の硫酸銅を添加して出現する新鮮イカ肝油の抗菌作用に対する阻害作用も上記酸化イカ肝油に対する阻害作用と略と同一であつた。

Table 2. Reversal of the inhibitory action of the autoxidixed calamary oil on *Debaryomyces membranaefaciens* by various substances (10 mg autoxidized calamary oil/10cc medium).

	Maximum amounts added	Growth of the yeast
Cholesterol	75 mg	−
Lecithine	75	−
α-Tocopherol	50	−
Cysteine	100	−
Glutathion	30	−
Na-thioglycollate	20	−
Activated charcoal	10	+
Starch	100	−
Medicinal yeast	100	+
Tsubaki oil	50	+
Stearic acid	50	−
Oleic acid	50	−
Biotin	1	−

考　　察

不飽和脂肪酸の抗菌作用には不飽和度が，飽和脂肪酸の抗菌作用には炭素数が大きな因子であり，不飽和脂肪酸では不飽和度の大きい程，飽和脂肪酸では C_8～C_{14} の脂肪酸が強力な抗菌作用を有することは既に報告されている[3)5)]。油脂の酸化により出現する抗菌力と基質の不飽和度との関係について Methyl oleate, linoleate 及び linolenate を酸化して追求したところ Table 3. に示した如く酸化により出現する抗菌力は基質の不飽和度と密接な関係があり不飽和度の大きいもの程抗菌力は大であつた。魚油の酸化により出現する抗菌作用と不飽和脂肪酸の抗菌作用とについて検討してみると，抗菌力が不飽和度に関係があること及び抗菌型式では極めて類似しているが，酸化イカ肝油の抗菌作用は Gram 染色性と無関係であること，不飽和脂肪酸に対する阻害剤である Cholesterol, Lecithine 及び α-Tocopherol 等により全く阻害されないこと及び不飽和脂肪酸の抗菌作用はエステル化することにより消失するといわれているが[3)]，酸化により出現する抗菌作用は Table 4. に示した如く酸化条件さえ同一であればイカ肝油でも，イカ肝油脂肪酸でもまたイカ肝油肪脂酸メチールエステルでも酸化により出現する抗菌力は等しかつたことから考えると酸化油の抗菌作用と不飽和脂肪酸の抗菌作用とは全く異つたものであり，Roth 等[18)]が不飽和脂肪酸は本来抗菌作用を有するものではなく不飽和脂肪酸の抗菌作用は総て実験中に不飽和脂肪酸が酸化して生じた酸化物によるものであると主張しているのは妥当でない。酸化イカ肝油の抗菌作用が前述の如く Cholesterol, Lecithine 及び α-Tocopherol の如き界面活性剤により阻害されないこと及び広範囲の菌種に作用すること等は飽和脂肪酸の抗菌作用と類似している点ではあるが，飽和脂肪酸の抗菌作用は培地の pH により影響されて酸性側に於て抗菌力が顕著に大であるといわれ，この原因について Karabinos 等[19)]は酸性に於ては脂肪酸が微生物或は微生物に必須の蛋白質と化学的に結合し易いためであると説明しているが，著者等の実験結果では

Table 3. Antibiotic activity of autoxidized methyl esters of unsaturated fatty acids on *Debaryomyces membranaefaciens*.

Methyl ester	Oleate	Linoleate	Linolenate
Antibiotic activity	Dilution units per gram of substance		
	100	200	400

Each of methyl esters was oxidized at 100°C for 1.5 hours by blowing oxygen in the presence of M/100 Cu-stearate.

Table 4. Production of the antibiotic action by the oxidation of calamary oil and fatty acids and methyl esters derived from it.

Oxidizing time (hours)	1.5	3	5	7
Calamary oil	1000	1000	400	400
Calamary oil fatty acids	1000	1000	400	400
Calamary oil methyl esters	1000	1000	400	400

Each sample was oxidized at 100°C by blowing oxygen in the presence of M/100 Cu-stearate.

Table 5. Growth of *Debaryomyces membranaefaciens* at various pH in the presence of the antibiotic autoxidized calamary oil.

Autoxidized calamary oil added (mg) \ pH	5.5	6.0	6.5	7.0	7.5	8.0
10	−	−	−	−	−	−
7.5	+	+	+	+	+	+
0	++	++	++	++	++	++

Table 5. に示した如く酸化イカ肝油の抗菌力と培地の pH との間にはさして密接な関係は認められず酸性に於て特に抗菌力が大であるという現象は現われなかつた。このことより酸化イカ肝油は飽和脂肪酸とも異つた抗菌作用機構によつて微生物に作用すると考えられる。次に酸化イカ肝油が界面活性剤として微生物や培地に作用して抗菌作用を示すことも考えられるがこのような性質のものであるならば培地に無機塩或は他の界面活性剤を添加して界面活性を変化させると抗菌力も変る筈であるが酸化イカ肝油の抗菌作用は培地に食塩を5%迄添加しても又 Tween 20, Cholesterol, Lecithine 及び α-Tocopherol の如き界面活性剤を添加しても抗菌力は全く変化しなかつたので界面活性剤として作用しているとも考えられない。

上述の諸点から考察すると酸化イカ肝油の抗菌作用は不飽和脂肪酸或は飽和脂肪酸の抗菌作用とは異つたものでありまた酸化に伴う界面活性に由来するものでもなくて，酸化により生成する是等と作用機構を異にする酸化生成物によると考えるのが妥当であらう。

総　　括

1. 酸化イカ肝油は細菌類に対しては Gram 染色性には殆ど無関係に作用し，抗黴性は一般に弱く或る種の真正酵母菌及び不完全菌に対しても抗菌作用が弱い場合もあつた。抗菌力は培地に微量の $CuSO_4$ (5×10^{-4}M) を添加して出現する新鮮イカ肝油の抗菌力と略々比例した。

2. 酸化イカ肝油の抗菌作用は主として誘導期の延長によるもので菌の発育初期に添加した場合に有効であつた。定常期に添加した場合には添加量によつては発育促進作用が表われた。

3. 酸化イカ肝油の抗菌作用は Cholesterol, Lecithine 及び α-Tocopherol の如き界面活性剤によつても，Sulfhydryl 化合物によつても阻害されず，植物油の不鹸化物，薬用酵母及び吸着剤としては活性炭によつてのみ阻害された。

4. 酸化イカ肝油の抗菌作用は不飽和脂肪酸，飽和脂肪酸及び界面活性剤とは抗菌作用機構を異にする酸化生成物によるものと考えられる。

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魚体成分の抗菌作用に関する研究—Ⅷ.
酸化魚油中の抗菌性物質の分別

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魚体成分の抗菌作用に関する研究—Ⅷ. 酸化魚油中の抗菌性物質の分別

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Studies on the Antibiotic Action of Fish Components—Ⅷ. Fractionation of an Antibiotic Substance from Autoxidized Fish Oils

Masamichi Toyomizu

In the present work, the stability of the antibiotic autoxidized fish oils and the fractionation of an antibiotic substance have been studied, and the following results were obtained.

1) The antibiotic autoxidized fish oils are not stable to thermal treatment by which further oxidation is promoted. They are also unstable to alkaline treatment, but stable to ultraviolet radiation.

2) An antibiotic substance could not be fractionated by the vacuum or steam distillation. The urea segregation has been found to be a useful technique for the fractionation of it.

3) An antibiotic substance, contained in autoxidized fatty acids or methyl esters derived from shark liver oils, was concentrated into petroleum ether soluble fraction which do dot form urea complex. Further purification was carried out by chromatography using activated charcoal and petroleum ether.

魚油が紫外線照射及びオゾン化により抗菌作用を現わし，この作用は過酸化物価と関係があることは既に報告され[1-3]，又最近不飽和脂肪酸の酸敗により生ずる抗菌作用は酸化により生ずる過酸化物に基くという主張がある[4,5]。一方 Ferlin 等[6] は Tall oil-ethyleneoxide condensate をオゾン化して得た Polyethenoxy tallate ozonide が抗菌作用を示し，この抗菌性物質を蒸気蒸溜により分離して Pelargonic acid CH_3-$(CH_2)_7$-COOH (B.P.250°C) であると報告し，更に油の酸化物からは Pelargonic acid が屢々検出されているので同氏等[7] は酸敗油の抗菌作用は油の酸敗により生成する Pelargonic acid によると述べている。

著者等[8] がイカ肝油を用いて行った酸化条件と抗菌作用出現に関する実験結果によると，過酸化物価が大となるような低温における酸化では出現する抗菌力は小であり，100°C に於て Cu-stearate を M/100 になる如く添加して乾燥酸素吹込み下で 1.5～3.0 時間加熱した場合が酸化油の抗菌力は最大であつて，この時の過酸化物価は他の状態における酸化に比し決して大なるものではなく，過酸化物価と抗菌力との間には直接的な関係は認められなかつた。

本報では抗菌性酸化油の安定性を明かにした後，水蒸気蒸溜法及び減圧蒸溜法により抗菌性物質の分別を試みた処酸化魚油の抗菌性物質を分離し得なかつた。しかし尿素附加物による分別を行つたところ，高濃度の抗菌性油状物質を得たのでその結果を報告する。

実 験 の 部

Ⅰ. 抗菌性酸化魚油の安定性

酸化魚油中の抗菌性物質を分離するに当り，操作を撰択する上にその物質の安定性を明かにすることが必要であると考え，先ず熱に対する安定度を酸化イカ肝油（抗菌力 1,000）を試料として試験した。その結果は Table 1. に示した如くであつて，抗菌性物質は明かに酸化過程に生ずる中間生成物であり更に酸化を進めるような条件下では不安定であつた。その他の場合では熱に対して比較的安定であつて特に減圧下及び炭酸ガス中では熱に対して安定であつた。又紫外線照射に対しても安定であつて石英水銀燈で 2 時間照射して

1954 年 2 月 7 日受理

も抗菌力は全く変化しなかつた。抗菌性酸化イカ肝油は等量の水と1時間煮沸しても抗菌力に変化はなかつたが，これを鹸化すると抗菌性を全く失つた。即ちアルカリに対しては極めて不安定で同量の 0.1 N NaOH と室温で 10 分間振盪する丈で抗菌力は半減した。然し乍ら酸に対しては安定であつた。

Table 1. The stability of the antibiotic autoxidized calamary oil on thermal treatment.

At 30°C.

days	0	10	25
In air	1,000	400	400
In carbon dioxide	1,000	1,000	1,000

At 100°C.

hours	1.5	3	4.5	6
Blowing of oxygen	1,000	400	400	200
In air	1,000	1,000	400	400
Under vacuum (5mm. Hg)	1,000	1,000	1,000	1,000
Blowing of carbon dioxide	1,000	1,000	1,000	1,000

At 200°C.

hours	0	0.5	2
In air	1,000	400	400
Under vacuum (5mm. Hg)	1,000	1,000	1,000

Ⅱ．酸化魚油より抗菌性物質の分別

前述の如く抗菌性物質は温度に対して安定であり減圧下においては 200°C で2時間抗菌力が変化しなかつたので減圧蒸溜による分離を試みた。蒸溜に当つてはグリセライドのまま分溜するよりも脂肪酸又は脂肪酸メチールエステルとして分溜するのが容易であり，是等3種の物質の酸化により出現する抗菌力には差異が表われなかつたので酸化イカ肝油及び酸化イカ肝油脂肪酸メチールエステルを用いて減圧蒸溜（5mm Hg）により分離を試みた。その結果前者では 63°C，後者では 60°C から溜出が始まりその時の金属浴の温度はそれぞれ 319°C，270°Cであつて溜出の最盛時には脂肪酸メチールエステルにおいても 300°C 以上であり，且つ各溜出区分はいづれも 200～1,000 の抗菌力を示した。上述のことと各区分の収量から考えて酸化により出現した抗菌性物質が溜出したのではなくて，むしろ加熱中に生成した物質が抗菌作用を示したものと思われる。又水蒸気蒸溜によつては抗菌性物質は全く溜出しなかつた。

最近不飽和脂肪酸の酸化生成物を尿素によつて分別する方法が報告されているが[9,10]，この方法と Bengen[11] が直鎖化合物が尿素と附加物を作ることを発見したことに始まり，油脂化学の分野では広く応用され，金田[12] は不飽和脂肪酸の毒性物質をこの方法により分別して尿素附加物を作らぬ部分に毒性が存在することを明かにした。又 Abu-Nasr 等[13] は魚油から高度不飽和脂肪酸を分別するに当り，魚油脂肪酸及びそのエチールエステルを試料とし，尿素を数回に分けて添加し尿素附加物を分別し高度不飽和脂肪酸の尿素附加物生成について難易を論じたが著者もこの方法に準じて（Fig. 1.）サメ肝油脂肪酸及びそのメチールエステル並びに常法[8] に

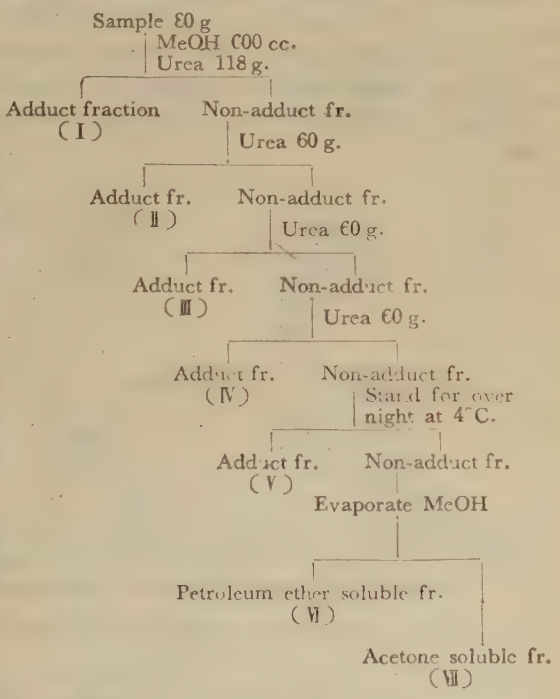

Fig. 1. Fractionation by the use of urea inclusion compounds.

より酸化した是等の酸化物（共に抗菌力 400）について分別を行つた。その結果尿素附加物からは試料 I 一 V を得，尿素附加物を作らぬ部分からは新鮮な未酸化の試料では石油エーテル可溶の試料 VI のみを，酸化した試料からは試料 VI 及びアセトン可溶部から脱水して得た試料 VII を得た。各区分について収量及び沃素価を，酸化した試料では是等の外に過酸化物価及び酸化の程度を知るために 230 mμ に於ける吸光係数を測定した。その結果は Fig. 2, 3. に示した。即ち新鮮サメ肝油脂肪酸及びそのメチールエステル（Fig. 2）では尿素附加物の生成状態は脂肪酸の場合とメチールエステルの場合とでは明かに異り，脂肪酸の場合では二重結合の多い部分は尿素附加物を作り難く，各区分は沃素価の順に配列し，沃素価の最も高い区分は尿素附加物を作らない区分（VI）であつた。然るにメチールエステルの場合は沃素価の高い区分も尿素附加物を生成し，沃素価の最も高い区分は尿素附加物を作らない区分（VI）ではなくて，4°C で析出した尿素附加物を形成する区分（V）であつた。是等は ABU-NASR 等の結果とよく一致している。

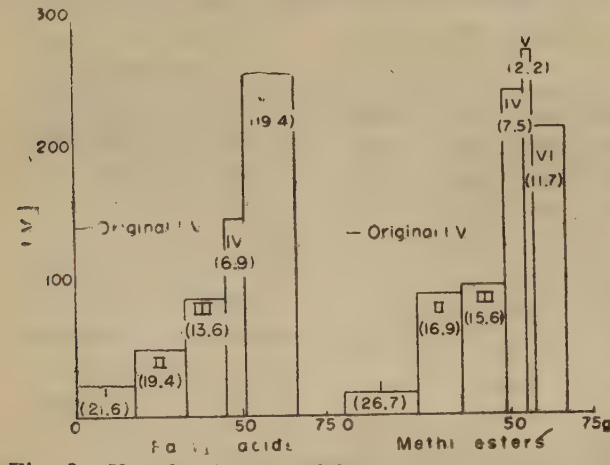

Fig. 2. Urea fractionation of fatty acids of shark liver oil and their methyl esters.

Numbers within bars refer to content (%) of fractions. Widths of bars indicate weights of fractions, heights indicate iodine values.

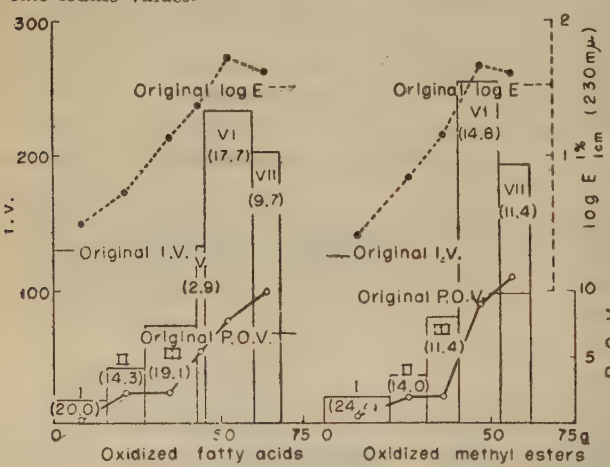

Fig. 3. Urea fractionation of fatty acids of autoxidized shark liver oil and their methyl esters.

Explanation of figure is the same as for Fig. 2, and circles indicate peroxide values (—o—) and log $E^{1\%}_{1cm}$ at 230 mμ (···•···).

然るに常法[3]で酸化を行つた酸化脂肪酸及び酸化脂肪酸メチールエステル（Fig. 3）では前の場合と趣を異にし，尿素附加物生成の難易は酸化による影響が大であつて，酸の型であるか，メチールエステルの型であるかという差異の影響は殆ど現れず，新鮮な場合に比して酸化物では尿素附加物は生成し難く尿素附加物を作らない区分（VI，VII）の収量が増大した。抗菌性物質は酸化脂肪酸でも酸化脂肪酸メチールエステルでも尿素附加物を生成しないで石油エーテルに可溶性の区分（VI）のみに濃縮され，抗菌性物質の分別にはこの両者の間に難易はなく，収量は前者で 17.7%，後者で 14.9 %であつて抗菌力は共に 2,000 であつた。

このものは青褐色の液体油でこの儘の状態では不安定であり，これを石油エーテルに溶かし室温に放置すると重合物と思われる褐色の石油エーテル不溶物を析出するに伴い抗菌力は漸減し，この現象は極小量のアンモニアの添加により促進された。この点から抗菌性物質は油が酸化して生ずる重合体の Precursor であるとも考えられる。

尿素附加物を生成しない石油エーテル可溶の抗菌性油では抗菌性物質は石油エーテルから 85 % エタノールに移行せず又活性アルミナによる Chromatography ではいづれの区分からも抗菌性物質は得られなかつた。これは吸着された抗菌性物質が溶出されないためと考えるよりも活性アルミナのアルカリ性反応のために抗菌性物質が不活性化されたと考える方が妥当であろう。

次に吸着剤として活性炭 Column(1.5

×13″) を用い，酸化サメ肝油脂肪酸から尿素法により分別した試料（抗菌力 2,000）3.6 g を 50 cc の石油エーテル（B. P. 40°～70°C）にとかし，300 cc の石油エーテルで溶出して Chromatography を行つて抗菌力 3,000 の微黄色の油状物質（沃素価 245.6，過酸化物価 28.4）1.7 g を得た。この物質の化学的性質等については後報する。

摘　　要

1. 抗菌性酸化魚油の安定性及び分別法について研究した。

2. 抗菌性酸化魚油は酸化を進めるような加熱及びアルカリ処理に対しては不安定であつたが，紫外線照射には安定であつた。

3. 抗菌性酸化魚油は減圧蒸溜及び水蒸気蒸溜によつては分別されなかつたが尿素法により分別された。

4. 抗菌性物質は尿素分別法によると酸化サメ肝油脂肪酸においても酸化サメ肝油脂肪酸メチールエステルにおいても尿素附加物を生成しない石油エーテル可溶部に濃縮され，この抗菌性物質は活性炭による Chromatography で精製された。

終稿に当り終始懇篤なる御指導を賜つた富安教授に謝意を表する。

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Studies onthe Antibiotic Action of Fish Components—IX. The Relation between the Antibiotic Action of the Autoxidized Shark Liver Oil and Thiobarbituric Acid Value

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Studies onthe Antibiotic Action of Fish Components—IX. The Relation between the Antibiotic Action of the Autoxidized Shark Liver Oil and Thiobarbituric Acid Value

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In the previous paper[1], it has been described that the antibiotic autoxidized fatty acids and methyl esters of shark liver oil are fractionated by means of urea. The need for an objective test which will correlate with the antibiotic action of the oxidized oil is apparent in this field.

Accordingly in the present paper, the properties of the concentrated antibiotic autoxidized fatty acids of shark liver oil were investigated and then the relation between the antibiotic action and certain oxidative products such as conjugated acid, peroxide, lactone, carbonyl compound etc. was discussed, in order to determine what group of the oxidative products is responsible for the antibiotic action.

Experimental

1. The properties of the concentrated antibiotic autoxidized acids of shark liver oil

The concentrated antibiotic autoxidized fatty acids of shark liver oil, obtained from non-comprex-forming fraction by means of urea and then purified by chromatography on activated charcoal as shown in the previous paper[1], had the antibiotic activity of 3,000. The iodine value and peroxide value (POV) of the fraction were 245.6 and 28.4, and these values were higher than the values of the original autoxidized fatty acids which were 128.4 and 7.0 respectively. The ultraviolet spectrum of the fraction measured in ethanol is shown in Fig. 1. The light absorption at 233 mμ due to the conjugated diene was high and the absorptions at 268 and 315 mμ region due to the conjugated triene and tetraene were low.

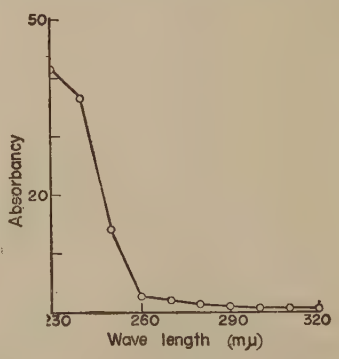

Fig. 1. Ultarviolet absorption spectrum of the antibiotic autoxidized fatty acids of shark liver oil which were fractionated by means of urea and purified by chromatography.

According to the GRAF's qualitative analysis[2]

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conducted by the hydroxylamine reagent, ketone and lactone or ester were detected but aldehyde and acid anhydride were not detected as shown in Table 1. However, since the

Table 1. GRAF's qualitative analysis of the antibiotic autoxidized fatty acids of shark liver oil which were fractionated by means of urea and purified by chromatography

	Ketone	Aldehyde	Acid anhydride	Lactone or ester
Concentrated antibiotic fatty acids	+	−	−	+

sodium nitroprusside reaction, which is to test aldehyde and ketone, was positive and since aldehyde-detecting reaction such as phenol-sulphuric acid reaction and fuchsin-aldehyde reaction also gave positive results, aldehyde is certainly present in the fraction.

2. The relation between the antibiotic action and the properties of the autoxidized shark liver oil

Conjugated acid : The antibiotic autoxidized fatty acids of shark liver oil concentrated by means of urea, contain a plenty amount of conjugated diene as shown by spectrophotometric method. In an attempt to solve the question whether the antibiotic action is due to conjugated acids or not, the content of diene of the fatty acids derived from shark liver oil, which were autoxidized at 100° by blowing dry oxygen in the presence of M/100 Cu-stearate according to the normal autoxidation procedure[3], was calculated out of absorption data, and then the relation between the production of conjugated diene and the antibiotic action was investigated, but any relation was not found at all (Table 2). Conjugated

Table 2. The relation between the antibiotic action and conjugated diene produced during autoxidation of the fatty acids of shark liver oil

Oxidation time (hr.)	0	1.5	3	5	7	9
Conjugated diene (%)	1.10	2.31	2.37	3.83	4.04	4.18
Antibiotic activity	0	400	500	400	200	200

triene and tetraene also increased progressively with the time of oxidation and the highest value of each was at 9 hr. oxidation just as the case of conjugated diene. Therefore these conjugated acids do not appear to be connected with the antibiotic action of the autoxidized oil.

Peroxide : Several investigators have shown that the antibiotic action and the toxicity of the oxidized oil for the growth of rat and chick are due to peroxide, and COLEMAN *et al.*[4] have reported that peroxide is concentrated in the non-complex-forming fraction of the oxidized unsaturated fatty acids fractionated by means of urea. POV of the concentrated antibiotic autoxidized fatty acids of shark liver oil, which are non-complex-forming fraction, is 28.4 and is relatively high compared with the value of the original oxidized fatty acids as described above. Moreover the antibiotic activity of the autoxidized oil disappeared with the intense decrease in POV after saponification. For these reasons the relation between the antibiotic action and POV during oxidation was investigated. POV increased progressively with the time of oxidation, showing no connection with the antibiotic action (Table 3), and the antibiotic activity of the oil autoxidized at 100° was higher

than that at 60° or 80° which is more suitable to produce plenty amount of peroxide than 100°. Moreover, pyridoxine and pantho thenate which are effective to eliminate the toxicity[5)6)], did not reverse the antibiotic action of the oxidized shark liver oil.

Lactone : Le GOUSSE[7)] showed that lactone and lactide are produced by the oxidation of unsaturated fatty acids, and it was reported by STEENSHOLT[8)] that lactone inhibits enzyme action. From the results of the qualitative analysis of the concentrated antibiotic autoxidized fatty acids derived from shark liver oil, it is possible that they contain lactone as described in this paper. Therefore the change of lactone content during oxidation of shark liver oil was measured. Since lactone will change to hydroxyl compound by saponification, the relation between lactone and the antibiotic action was investigated by the estimation of acetyl values before and after saponification.

The antibiotic autoxidized shark liver oil is unstable for alkali treatment as shown in part VIII[1)], and the antibiotic activity disappeared completely after saponification, hence the relation was sought between the antibiotic action and the values for the alkali reagent, such as acid value and saponification value for the autoxidized shark liver oil and neutralization value for the autoxidized fatty acids of the shark liver oil. These results are shown in Table 3. Acetyl values before saponification did show a continuous increase with the time of oxidation and they were still quite low, whereas the corresponding acetyl values after saponification increased considerably during oxidation and were always higher than those before saponification, possibly suggesting that lactone is produced with the time of oxidation and has no correlation with the antibiotic action. The changes of acid-, saponification- and neutralization-value also did not correlate with the antibiotic action.

Table 3. The relation between the antibiotic action and variable value of the autoxidized shark liver oil

Oxidation time (hr.)		0	1.5	6
Peroxide value	Before saponification	2.20	6.68	9.52
	After saponification	0.80	0.34	0.90
Acid value		2.61	3.01	4.55
Saponification value		190.2	197.1	205.1
Neutralization value (Fatty acids)		194.2	186.0	176.5
Acetyl value	Before saponification	4.33	7.71	7.81
	After saponification	8.31	14.43	25.25
Antibiotic activity		0	400	200

Peroxide value : ml. of 0.01 N $Na_2S_2O_3$ soln./gr.

Carbonyl compound : Since aldehyde and ketone are present in the concentrated antibiotic autoxidized fatty acids of shark liver oil as described above, total carbonyl fat was estimated by the procedure proposed by HENICK *et al.*[9)], which is based upon the colorimetric determination of the hydrazone compounds in alkaline solution and the calculation from the absorbancies at 430 and 460 mμ after preparing 2,4-dinitrophenylhydrazones of carbonyl fat. Total carbonyl fat of the autoxidized shark liver oil and the antibiotic action decreased extremely after saponification, but the former increased during oxidation, having no connection with the latter (Fig. 2).

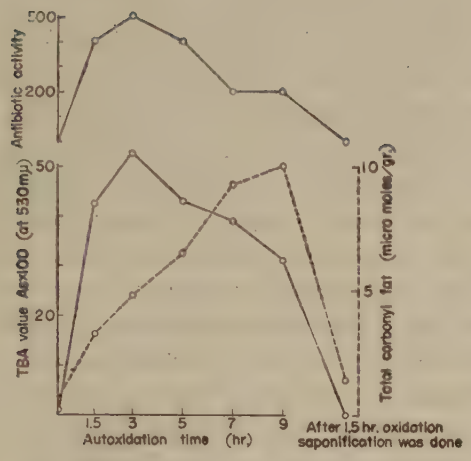

Fig. 2. The relation between the antibiotic action and TBA value or carbonyl fat content of the autoxidized shark liver oil.

Recently the rancidity of oil and fat has been estimated by the color reaction with thiobarbituric acid (TBA). In 1944 KOHN and LIVERSEDGE[10] showed that tissue suspensions of heart and liver incubated aerobically at 30° produce a compound which will condense with either *p*-aminobenzoic acid to give a yellow-color or with TBA to give an orange-color. Subsequently BERNHEIM *et al.* concluded that the color reaction is due to fat oxidation in tissue[11][12] and that the inhibitory action of the oxidative products of unsaturated acid on certain enzme action is proportional to the color reaction[13][14]. The author investgated the relation between TBA value and the antibiotic action, measuring TBA value according to the TBA acetic acid procedure modified by SIDWELL *et al.*[15] It can be observed in Fig. 2 that the TBA curve has the same characteristics as the curve for the antibiotic activity, namely both values were maximum at 3 hr. oxidation, gradually decreased by the further oxidation and disappeared by saponification (Fig. 2).

Discussion

It has been reported that TBA reacts on aldehyde and ketone, giving orange-colored products[16][17][18]. PATTON *et al.*[19] used the color reaction for the estimation of rancidity of milk fat and described that malonic dialdehyde in rancid fat gives strong color. KENASTON *et al.*[20] also concluded that aldehyde produced by fat oxidation condenses with TBA and that the condensed products are tinged with orange-color.

If all carbonyl compounds in oxidized oil were tinged with the color in the presence of TBA and same color intensity appeared, the absorption at 530 mμ would have to change in parallel with the content of total carbonyl fat. However, parallel change was not observed as shown in Fig. 2. It means that the reaction does not proceed as described above. SEDWELL *et al.*[15] have also described that the color intensity of the oxidized oil with TBA has no connection with the content of total carbonyl fat.

As BUSS and MACKINNEY[21] have isolated thirty 2,4-dinitrophenylhydrazones of carbonyl compounds from rancid corn oil, carbonyl compounds produced during oxidation seem to be numerous. The antibiotic action of the autoxidized oil had close relation with the color reaction not connecting with the content of total carbonyl fat (Fig. 2). This suggests that a special carbonyl compound produced during oxidation probably inhibits microbial growth. Since the antibiotic autoxidized fatty acids, concentrated by means of urea, are present in non-complex-forming fraction and this fraction is unsaturated fraction as described in part

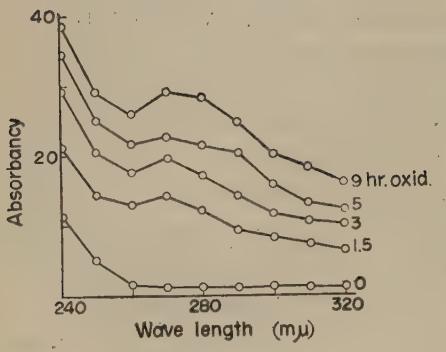

Fig. 3. Ultraviolet absorption spectrum of the autoxidized fatty acids of shark liver oil in alkaline ethanol solution.

VIII[1], the antibiotic substance in the autoxidized fatty acids seems to be an unsaturated carbonyl compound.

Ultraviolet absorption of the oxidized fatty acids when measured in ethanol is due to conjugated acids produced by isomerization during oxidation. On the other hand, when the oxidized oil is dissolved in an alkaline medium, intense orange- or red-color is produced. This is the result of the production of chromophores whose principal absorption lies in the ultraviolet range. The alkaline color has been studied spectrophotometrically by HOLMAN *et al.*[22] and it was concluded that the color was probably not due to quinones formed from dicarbonyl compounds in the oxidized oil. His present opinion[23] is that the color is due to condensation of unsaturated carbonyl compounds. Therefore, ultraviolet absorption spectrum of the autoxidized fatty acids derived from shark liver oil, dissolving in the solution of 20 % potassium hydroxide solution and ethanol (1 : 1), was measured and the result is shown in Fig. 3.

The absorption at 240~320 mμ increased with the time of oxidation as the case of absorp tion in ethanol, and the value connected with the antibiotic action was not obtained by this method.

Summary

The antibiotic action produced by the autoxidation of shark liver oil correlated with thiobarbituric acid value, having no connection with total carbonyl fat content and peroxide value. Furthermore, the antibiotic fraction concentrated by means of urea was non-complex-forming fraction, so the antibiotic substance is presumed to be an unsaturated carbonyl compound.

Acknowledgment

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A new species of copepod, *Scolecithricella vespertina*, .sp. from the Sea of Japan*

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A new species of copepod, *Scolecithricella vespertina*, .sp. from the Sea of Japan*

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Female. Length, 1.09 mm. The Cephalothorax oval. The Head fused with the 1st thoracic segment. The frontal margin of the head narrory rounded. The 4th and the 5th thoracic segments are fused. The lateral margin of the last thoracic segment is slightly emarginate. The Rostrum consists of two fine filaments attached to a wide difurcated base.

The Abdomen 4-Jointed, and is contained about 4-times in the length of the cephalothorax. The abdominal segments and furca are in the proportional lengths:

1+2	3	4	5	Furca
40	20	17	6	17 =100

The furcal rami 1.5 -times as long as wide. The genital segment is slightly swollen at the middle of the lateral margin when viewed from the dorsal; the ventral surface of the segment slightly produced below.

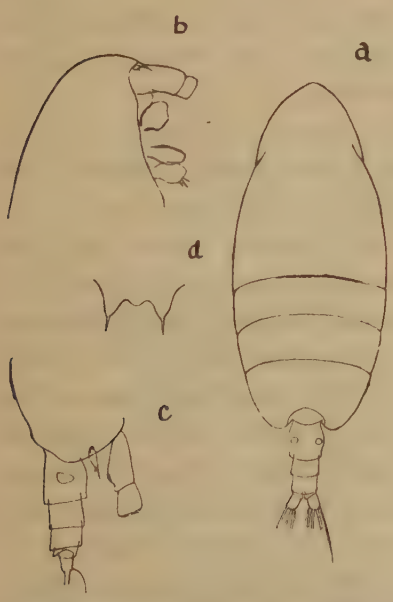

Fig. 1. a, body, dorsal view, X26; b, head, lateral view X 33; c, last thoracic segment and abdomen, lateral view, X 33; d, rostrum, X 131.

Fig. 2. a, 2nd antenna, X131; b, mandible, X131; c, 1st maxilla, X131.

The 1st antenna 21-jointed, extends to the posterior margin of the last thoracic segment. The joints 1 and 2, 8 to 10, and 24 and 25 are fused. The joints 12 and 13 are fused on the posterior margin. The joints are in the following propotional lengths:

1-2	3	4	5	6	7	8-9-10	11	12	13	14	15	16	17	18	19	20	21	22	23	24-25
99	25	25	25	25	25	64	25	25	29	39	44	44	49	49	49	49	54	64	84	108=1000.

The 2nd antenna well developed. The exopodite is adout 1.3-times (37 : 28) as long as the endopodite. The 2nd joint of the endopodite is swollen on the outer margin, and is furnished with hairs on the outer margin. The Mandible small, but well developed. The exopodite about 2-times as long as the endopodite (11 : 6). The 3rd joint of the exopodites the longest (8 : 7 : 12 : 3 : 2). The teeth of the cutting edge are slender. The Maxilla is small. The spinulation on the various parts are as follows: the outer lobe, 6 long and 3 short setae; the exopodite, 5 setae; the endopodite, 6 setae; the 2nd basal joint, 2 setae; the 3rd inner lobe, 2 setae; the 2nd inner lobe, 1 seta; the 1st inner lobe, 8 long and 1 short spines. The 2nd maxilla has 4 inner lobes, of which the distal one is the strongest; it is furnished with a strong spine, a slender one and a wormlike sensory filament. The endopodite of the 2nd maxilla is furnished with 9 sensory filaments of which the middle 4 are bud-like. The Maxillipede well developed. The basal joints are of about equal lengths. The 1st basal joint has a bud-like sensory filament on the inner margin about the middle of the joint. The 4th and the 5th joints of the endopodite have each an outer marginal seta.

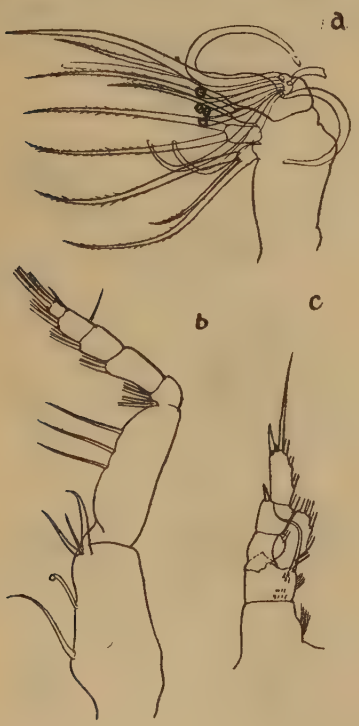

Fig. 3. a, 2nd maxilla, X131; b, maxillipede, X131; c, 1st leg, posterior aspect, X52.

The 1st leg has 3-jointed exopodite and 1-jointed endopodite. The 1st joint of the exopodite is devoid of outer edge spine. The 2nd basal joint is furnished with two groups of minute spinules on the posterior surface. The 2nd leg has 3-jointed exopodite and 2-jointed endopodite. The 1st basal joint of the 2nd leg has small spines on the proximal outer margin, and a row of minute spinules on the posterior surface about the middle of the joint. The inner distal margin of the 2nd basal joint is produced into an acute angle. The 1st joint of the exopodite has the outer edge spine reaching the middle of the 2nd joint of

the exopodite. The posterior surface of the joint is furnished with a longitudinal row of spinules. The 2nd joint of the exopodite has a strong outer edge spine which extends to the middle of the proximal outer edge spine of the 3rd joint. The posterior surface of the joint is furnished with two rows of spinules. The 3rd joint of the exopodite has 3 outer marginal spines. The terminal spine is finely serrated; the teeth are about 40 in number. The posterior surface of the 3rd joint is furnished with 4 transverse rows of spinules as shown in the figure. The endopodite is broad The 1st joint is pointed at the distal outer edge. The 2nd joint has 6 remarkable spines on the anterior surface and a longitudinal row of minute spinules on the posterior surface. The 3rd leg has 3-jointed exopodite and endopodite. The 1st basal joint has a row of spines on the proximal outer margin; the posterior surface is furnished with groups of spinules; the inner marginal seta short. The 2nd and 3rd joints of the exopodite are each furnished with rows of minute spinules on the posterior surface. The 2nd and 3rd joints of the endopodite are each furnished with remarkable spines on the anterior surface, and with a longitudinal row of minute spinules on the posterior surface as shown in the figure. The 4th leg has the similar structure as in the 3rd leg. The outer margin of the 1st basal joint smooth. The inner marginal seta absent. There is on the posterior surface a row of strong spines, 5 in number, at the distal margin of the joint, and a group of minute spinules about the middle of the joint. The 2nd joint of the exopodite has a very short outer edge spine and a transversal row of minute spinules on the posterior surface. The 3rd joint of the exopodite has 3 rows of minute spinules on the posterior surface. The terminal spine is very finely serrated. The 2nd and 3rd joints of the endopodite are each furnished with longitudinal rows of spinules on the posterior surface. The 5th leg is 1-jointed. The inner margin has on the middle of the joint a serrated spine which is about half as long as the joint. The distal margin of the joint is furnished with a short spine. The outer margin has a small spine on the distal one-fourth of the joint.

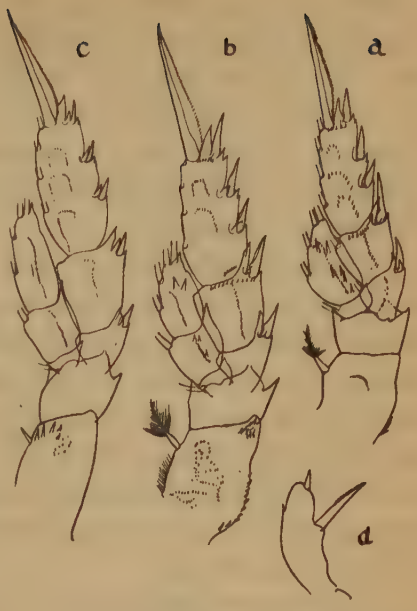

Fig. 4. a, 2nd leg, anterior aspect, X 52; b, 3rd leg, posterior aspect, X 52; c, 4th leg, posterior aspect, X 52; d, 5th leg, X 131.

Mori (1937) recorded the occurrence of th following species from the adjacent waters of Japan: *Scolecithricella minor* (Brady), *S. bradyi* (Giesbrecht), *S. abyssalis*

(Giesbrecht), *S. orientalis* Mori, and *S. spinipedata* Mori. These were taken chiefly from the surface layers. Among those *S. minor* is the cold water species, and has been recorded from the northern waters; the remainings have been reported from the warm waters. *S. orientalis*, though I have not found it in my collections, appears to be valid. But *S. spinicauda* seems to be identical with *S. ctenopus* (Giesbrecht) in having the 2nd, 3rd, and 5th pair of legs quite similar in structure to those of *S. ctenopus*.

The writer (1953) has reported 23 species of the genus Scolecithricella from the Pacific coast of the Middle Japan. Most of them appear to be bathypelagic, except *S. bradyi* and *S. ctenopus* which have been taken from the surface in the region. It is astonishing that the night haul made in the surface layer of the Japan Sea by the Kyoto Fisheries Experimental Station in July of 1953 caught *S. beata* which was one of the deep-water species of the Pacific, and was described by the writer (the description and figures yet unpublished). The present species may probably be an inhabitant of deep layers, and must have come up to the surface in the evening.

The specimen, though much smaller in size than *S. valida* (Farran), has the The 5th pair of legs quite similar in structure to those of *S.valida*. However, the spinulation on the 1st basal joint of the 3rd and 4th legs are quite different from those of the other species which have hitherto been observed by the writer.

Two specimens was taken in the surface night hauls in July of 1953 at the station 40 miles north of Maizuru.

*Foot Note. This paper is a result of the study carried out by the financial aid of grant from the Scientific Research Fund of the Ministry of Education.

THE PELAGIC COPEPODS OF THE IZU REGION, MIDDLE JAPAN
SYSTEMATIC ACCOUNT I.
FAMILIES CALANIDAE AND EUCALANIDAE

Otohiko TANAKA

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SYSTEMATIC ACCOUNT I.
FAMILIES CALANIDAE AND EUCALANIDAE

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With 5 Text-figures

The report had in the original manuscript the following contents: Introduction, Material and method, List of stations and date of collection, List of species, Systematic account, Zoogeographical and vertical distribution of the species, and Reference list. The first three chapters have been published in the journal "Records of Oceanographic Works in Japan, New Series, vol. I, no. 1, pp. 124–137, 1955." The present paper deals only with the Systematic account and forms Part I of the series which will be appeared continually in this journal. The present study was completed in 1948, but owing to publication difficulties the appearance of the paper was delayed much.

Systematic Account

Family CALANIDAE

Genus *Calanus*, Leach, 1819

The genus *Calanus* (sensu lato) has often been reduced by the separation of a number of species to other genus. At present the following genera are represented in the family Calanidae: *Calanus* Leach, *Calanoides* Brady, *Canthocalanus* A. Scott, *Neocalanus* G. O. Sars, *Megacalanus* Wolfenden, *Bathycalanus* G. O. Sars, *Undinula* A. Scott and *Nannocalanus* G. O. Sars.

These genera resemble so closely one another in general appearance that some of the authors still include them in the genus *Calanus*. *Calanus finmarchicus* (Gunnerus) is regarded as the type of the genus *Calanus*. The generic characteristics of *Calanus* in the restricted sense are as follows: Head is, in most cases, separate from the 1st thoracic segment; the 4th is separate from the 5th; the rostrum consists of two slender filaments; the 1st antenna and mouth parts are of normal structure; the 1st to 4th legs with 3-jointed exopodite and endopodite; the distal joint of the exopodite of the 1st to 4th legs with two outer marginal spines; the male 5th pair of legs have general

resemblance to those of the female, but the left leg is slightly modified in shape and armature.

Six species belonging to the genus *Calanus* (sensu stricto) have been recorded from the neighbouring waters of Japan: *C. finmarchicus* (GUNNERUS), *C. hyperboreus* KRÖYER, *C. tenuicornis* DANA, *C. cristatus* KRÖYER, *C. minor* (CLAUS) and *C. plumchrus* MARUKAWA. The last one has been reported from the northern water and is very closely allied to *C. tonsus* BRADY, some author distinguishes the species from the typical *tonsus* under a variety name, *C. tonsus* var. *plumchrus*. *C. hyperboreus* KRÖYER has been recorded from the Japan Sea by MARUKAWA, but I have not met with the species in the Izu region.

Calanus finmarchicus (GUNNERUS)

(Fig. 1, *a–j*)

C. finmarchicus GIESBRECHT, 1892, p. 89, t. 6, 7, 8; G. O. SARS, 1903, p. 11, pl. 4; ESTERLY, 1905, p. 125; WITH, 1915, p. 10; FARRAN, 1929, p. 212; WILSON, 1932, p. 23; *C. helgolandicus*, WILSON, 1932, p. 25; *C. finmarchicus*, JESPERSEN, 1934, p. 10; MORI, 1937, p. 13, pl. 2; *C. helgolandicus*, MORI, 1937, p. 14, pl. 1; *C. finmarchicus* SEWELL, 1947, p. 13; MARSHALL and ORR, 1955, p. 1; *C. helgolandicus*, MARSHALL and ORR, 1955, p. 4.

It has been noticed by many authors that there are two size groups in *C. finmarchicus*. According to SARS the large specimen is *C. finmarchicus*, and the small specimen *C. helgolandicus*. The size given by the authors are as follows:

	Female, Length (mm)	Male, Length (mm)
GIESBRECHT ·····	2.7–4.5	2.35–3.2
G. O. SARS ·····	about 4.0	3.60
	(about 3.0	2.80 *helgolandicus*)
WITH ·····	2.8–5.5	3.0–4.0
FARRAN ·····	2.64–3.2	
JESPERSEN ·····	3.5–4.5 (large size)	
	2.4–4.5 (small size)	
MORI ·····	about 4.0–5.0	about 3.6
	(about 3.0	about 2.8 *helgolandicus*)

The specimen of the Izu region measured:

3.5–4.07 (large size)	3.0–3.7
2.38–3.10 (small size)	2.45–2.80

SARS (1903) described a species of *Calanus* which he thought to be identical with the specimen reported by CLAUS under the name *Cetochilus helgolandicus*. He pointed out that the specimen differs from *C. finmarchicus* in its size, the proportional length of the furcal ramus, and in the structure of the male 5th legs.

The specimens of the Izu region, so far as I have examined, can be divided into

two size groups. These forms are found commonly in the surface and intermediate layers. The large form has the forehead more evenly rounded; the proportional lengths of the metasome to urosome 3.8 : 1; the furcal rami about 1.3 times as long as wide. In the small form the head is more produced and narrowly rounded; the proportional lengths of the metasome to urosome are 3.6 : 1; the furcal rami about 1.4 times as long as wide. The male of the small form has the left 5th leg with a

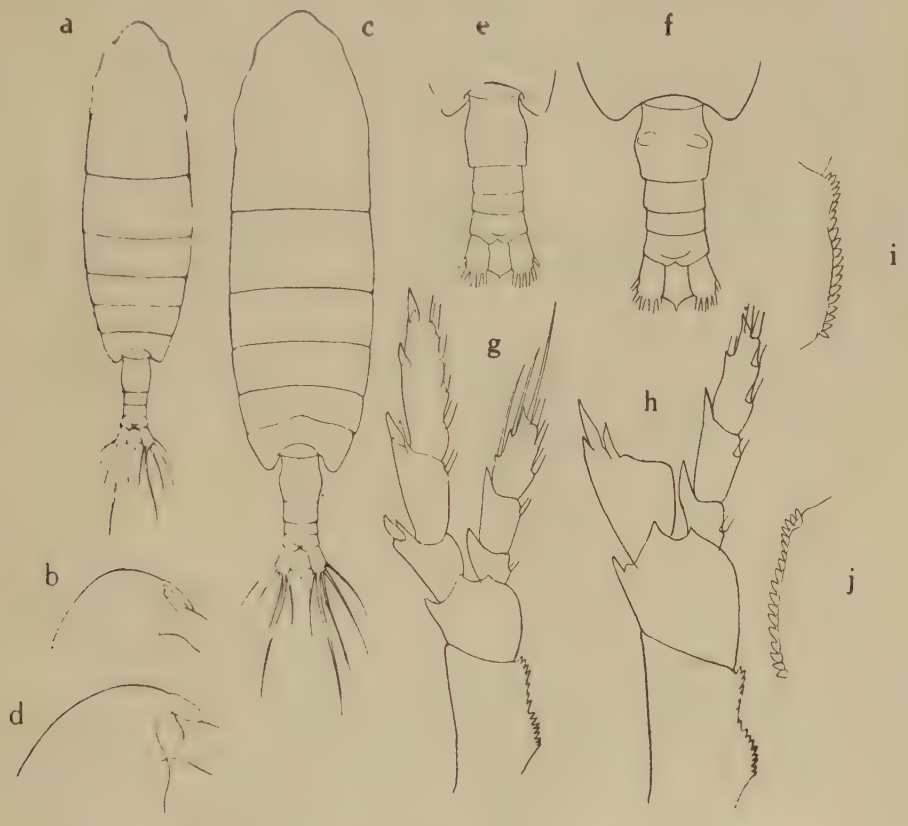

Fig. 1. *Calanus finmarchicus* (GUNNERUS).

a, Female: small specimen, dorsal aspect; *b*, head, lateral aspect; *c*, large specimen, dorsal aspect; *d*, head, lateral aspect; *e*, abdomen, dorsal (specimen 2.4 mm); *f*. abdomen, dorsal (specimen 2.9 mm); *g*, 5th leg (2.4 mm); *h*, 5th leg (2.9 mm); *i*, left inner margin of 1st basal joint of 5th leg (3.78 mm); *j*, right inner margin of 1st basal joint of 5th leg (3.78 mm).

slender exopodite than that of the large form. These differences are quite similar to those found in the specimens of the Europian waters. One could have, if he wishes, regarded the small form as *C. helgolandicus*, and the large as *C. finmarchicus*. MARSHALL and ORR (1955) report that there are two forms also in the Plymouth and Clyde sea area, though *helgolandicus* is larger than *finmarchicus* in those areas.

It has been reported that the size of *C. finmarchicus* shows a remarkable regional and seasonal variation. According to BOGOROFF the copepods grow larger in cold waters than in the warm. He shows as an example:

Locality	Average total length (mm)	Temperature °C
69°30′ N–75°N	3.22	4.6
75° N–77°N	3.78	1.08
North of 77°N	4.57	

JESPERSEN gives the following example:

Eastern North Sea	ca. 1.9–2.7 mm
West Greenland water	2.4–4.5

RUSSELL (1935) shows that in Plymouth area there are three main breeding seasons, and each brood differs in size. The individuals of the first brood during the cold period of the year grow to the largest, thereafter the remaining broods decrease in size as the temperature rises. The seasonal variation in size of the adult in the Izu region are as follows:

Month	Total length (mm)	
	Female	Male
December, 1936	2.38–3.01	2.56–2.98
February, 1937	3.75–3.86	
March, 1937	3.65–3.80	
April, 1937	4.03	3.49–3.70
June, 1937	2.80–2.90	

These two forms have each different habit in the vertical distribution; the small predominates in the upper layer down to 200 m, whereas the large in the layer deeper than 200 m; the immature specimens of the large form were found below 400 m in the month October 1937. The temperature of the 200 m layer was about 12°C, and that of the 400 m was about 8°C respectively. MORI reported that he has obtained only a single specimen of *C. finmarchicus*, but plenty of *helgolandicus* from Japanese waters. It can be attributed to the fact that he has collected only in the upper layer less than 200 m in the warm sea area.

As above mentioned, *C. finmarchicus* varies in size according to the conditions of life. The proportional lengths of the metasome to urosome show some individual difference. But there are some minute points of difference between these two forms. For instance, the inner margin of the 1st basal joint of the 5th legs of the female is straight or slightly convex in *finmarchicus*, whereas it is concave in *helgolandicus*. These two forms are, indeed, distinguishable, but there is no sufficient ground to raise the small form to the rank of species. Here I propose to separate the specimen with produced head from the specimen with evenly rounded head under a variety name *C. finmarchicus* var. *helgolandicus*.

Female. Length, large form, 3.50–4.07 mm. The abdomen is contained 3.6–4.1

times in the length of the cephalothorax; the genital segment is wider than long (36 : 32); the furcal rami about 1.3 times as long as wide (16 : 12); the 1st antenna extends to the end of the furca; the inner margin of the 1st basal joint of the 5th leg had is straight or slightly convex, and had 20 to 22 teeth.

The small form measured 2.38 3.10 mm; the cephalothorax narrow when viewed from the dorsal; the head is more produced; the abdomen is contained 3.5-4.2 times in the length of the cephalothorax; the genital segment is longer than wide (23 : 21-22); the furcal rami 1.4 times as long as wide (11 : 8); the 1st antenna exceeds the end of the furca by terminal two joints; the inner margin of the 1st basal joint of the 5th leg is straight or slightly concave, and had 14 to 22 teeth. The small specimen measured 2.90 mm had only five setae on the distal joint of endopodite of 5th pair of legs.

Male. Length. The large form, 3.49–3.70 mm; the small form, 2.50–2.98 mm; both forms have the 5th pair of legs of the similar structure except that the small form has the exopodite in the right leg reaching the proximal one-third of the 2nd joint of the exopodite of the left leg, whereas the large has the exopodite reaching the middle of the 2nd joint of the exopodite of the left leg.

Occurrence. Two forms are found commonly in the Izu region. The small form abundant in the upper layer, and the large predominates in the deeper layers.

Distribution. The species has a wide distribution in all oceans.

Calanus tenuicornis (DANA)

Calanus tenuicornis GIESBRECHT, 1892, p. 90. t. 6, 7, 8; A. SCOTT, 1909, p. 9; FARRAN, 1929, p. 217; SEWELL, 1929, p. 20; FARRAN, 1936, p. 77; MORI, 1937, p. 16, pl. 3, 4; VERVOORT, 1947, p. 22.

SARS included this species along with *C. gracilis* DANA and *C. robustus* GIESBRECHT in his proposed genus *Neocalanus*. But the absence of the hook on the 2nd basal joint of the 1st leg, and the fact that the head and the 1st thoracic segment are separate in this species, whereas in *gracilis* and *robustior* they are fused, are the sufficient grounds for separating the present species from the other too.

Length. Female, 1.99–2.10 mm. Male, 1.77 mm.

Occurrence. Three adult female and two males in a vertical haul from the depth 1000 m to the surface in Suruga Bay. The species appears to be rather a deep water species. It has been seldom obtained from the surface in the Izu region.

Distribution. The species is widely distributed in the tropical and subtropical regions of the Pacific, Atlantic and Indian Oceans.

Calanus plumchrus MARUKAWA

(Fig. 2, *a–d*)

C. plumchrus MARUKAWA, 1921, p. 10, pl. 1; *C.* sp. SATO, 1913, p. 3, pl. 2.
C. tonsus CAMPBELL, 1930, p. 177; TANAKA, 1954, p. 29, figs. 1–4.

C. tonsus MARSHALL and ORR, 1955, p. 8.
C. plumchrus TANAKA, 1956, p. 49, figs. 1–4.

Calanus tonsus BRADY was described by BRADY from the material taken in the southeast coast of the Australia (lat. 34°8′) and the east coast of South America (lat. 42°43′ S.). The species was also recorded by BRADY between Yokohama and Sandwich Island (Lat. 35°41′ N.). FARRAN recorded the species at stations off New Zealand between lat. 30°–40° S. and lat. 50°–60° S. WILSON recorded it from the

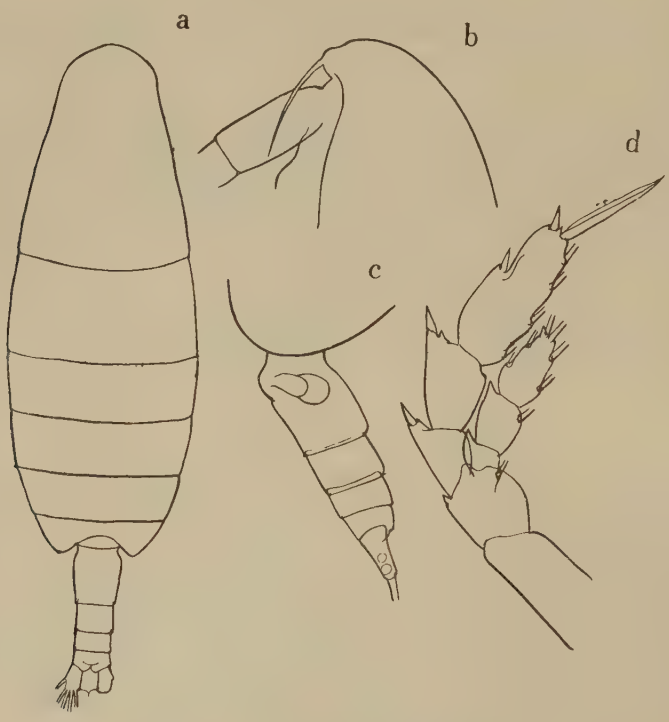

Fig. 2. *Calanus plumchrus* MARUKAWA.
a, Female, dorsal aspect; *b*, head, lateral aspect; *c*, abdomen, lateral aspect; *d*, 5th leg,

Woods Hole region. The occurrence of the species in the northern Pacific was reported by JOHNSON (1932), CAMPBELL (1934), TANAKA (1954), VINOGRADOV (1955), and BRADSKY (1955).

Recently Mr. J. NAKAI of the Tôkai Regional Fisheries Laboratory has informed me by personal communication that the original specimen of *C. tonsus* BRADY is not identical with the specimen from the northern Pacific. He says that the specimen from the southern hemisphere is smaller in size, and has the 2nd to 5th swimming legs which are furnished with small spines on the inner distal margin of the 2nd

basal joint; the proportional lengths of the proximal and distal outer margin of the 3rd joint of the exopodite divided by the outer marginal spine in the 2nd to 4th legs are different from those found in the northern specimen. These characters were mentioned by F. DAHL (1894) and by N. WOLFENDEN (1908) but not by BRADY. He must have overlooked these characters or examined the specimen which has no such characters. Accordingly there are forms in *C. tonsus* BRADY. In my previous paper I have made no distinction between the northern and southern specimens. Russian zoologists are of opinion that these forms are not distinct. MARSHALL and ORR (1955) distinguished the northern form from the southern one under a variety name *C. tonsus* var. *plumchrus*, but it is not clear upon whose reference they have made the distinction. NAKAI insists on the validity of *C. plumchrus* MARUKAWA. Recently I had an opportunity to examine the Antarctic specimen of *C. tonsus* BRADY. On comparing the specimen with that of the North Pacific, these two specimens are not identical. The specimen of the Antarctic is *C. tonsus* BRADY, and the specimen from the North Pacific is *C. plumchrus* MARUKAWA.

Female. Length, 4.83–6.32 mm; cephalothorax, 3.75 mm; abdomen, 1.08 mm; so the abdomen is contained 3.5 times in the length of the cephalothorax. The head separates from the 1st thoracic segment; the lateral corners of the last thoracic segment rounded; the rostrum with two long and slender filaments.

The abdomen 4-segmented, the joints and furca in the proportions 42 : 18 : 10 : 10 : 20 -100; the genital segment inflated at the proximal lateral margins; it is slightly longer than wide (42 ; 39) ; the furcal rami 2-times as long as wide.

The 1st antenna broken off in the distal joints; specimen measuring 5.90 mm had the joints in the proportional length in 0.01 mm:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
30	27	15	16	18	18	19	15	15	20	21	27	27	30	34	35	35	33.

The 2nd antenna has the endopodite about as long as the exopodite (50 : 48). The mandible has the exopodite 1.4 times as long as the exopodite (20 : 14). In the 1st maxilla the 1st inner lobe has no setae; the 2nd lobe has one or two setae; the 3rd has four long setae; the 1st outer lobe has six long setae and one short spine; the exopodite has ten setae; the distal joint of the endopodite has six setae, the 2nd joint has and 1st joint of the endopodite has four setae respectively; the 2nd basal joint has four setae. The 2nd maxilla feeble. The maxillipede soft-skinned and fairly reduced.

The 1st to 5th legs have each 3-jointed exopodite and endopodite. The outer margin of the distal joint of the exopodite of the 2nd and 4th legs is divided by the outer marginal spine into the proportional lengths 20 : 20, 26 : 21. The 5th leg has no inner marginal seta on the 1st basal joint, and the inner margin of the joint smooth; the distal joint of the endopodite bears six marginal setae; the outer margin of the 3rd joint of the exopodite is divided by the outer marginal spine in the proportional lengths 19 : 14.

Male. Length, 4.40–4.82 mm; the male specimen was fully described in my previous paper.

Remarks. The present specimens are larger in size than *C. tonsus* BRADY originally described by BRADY (3.60 mm). FARRAN's specimen from the South Atlantic measured 3.48–3.60 mm, and has the lateral margins of the genital segment of the female, when viewed from the dorsal is more inflated than those of the present specimen (FARRAN, 1929, fig. 12).

Occurrence. Twenty females in the vertical hauls from 1000 m to the surface in Sagami Bay, November 1937. The adult mole and female have been collected also from the deep waters of the Japan Sea.

Distribution. The northern form is widely distributed in the North Pacific. The adult specimen was found in Japanese waters from the deep layers of the Pacific and the Sea of Japan.

Calanus cristatus KRÖYER

Calanus cristatus GIESBRECHT, 1892, p. 91, t. 6; VAN BREMEN, 1906, p. 10; WITH, 1915, p. 11; MORI, 1937, p. 15; TANAKA, 1938, p. 599, fig. 1, 2.

The adult specimens, both female and male, have been obtained from the deep water of Sagami Bay in October 1938. Dr. M. W. JOHNSON informed me that he had also taken the adult specimens in the cold water of the North Pacific. The immature specimens has been reported from the surface water of northern Japan. The immature specimens were found in Sagami Bay from the intermediate layers between 300–500 m, whereas the adult from the depth more than 500 m.

Calanus hyperboreus KRÖYER

Calanus hyperboreus GIESBRECHT, 1892, p. 91, t. 6, 7, 8; G. O. SARS, 1903, p. 12; MARUKAWA, 1928, p. 21; WILSON, 1932, p. 21, fig. 8; JESPERSEN, 1934, p. 34.

The species has been recorded from the northern waters of the Atlantic. MARUKAWA recorded its occurrence from the Japan Sea, but it seems to be doubtful. According to SARS, the species has been found occasionally off the Finmark coast and Lofoten Island, together with *Calanus finmarchicus*, but further southwards it appears to be wholly restricted to the great depth of the fjords. WILSON records it from the Woods Hole region and the Gulf of Maine, and further south to the Gulf Stream in winter season. JESPERSEN recorded it from the Davis Strait and Baffin Bay.

Calanus minor (CLAUS)

Calanus minor GIESBRECHT, 1892, p. 33, t. 6, 7, 8; ESTERLY, 1905, p. 126; A. SCOTT, 1905, p. 7; *Nannocalanus minor* G. O. SARS, 1925, p. 9; SEWELL, 1929, p. 20; *Calanus minor*

WILSON, 1936, 2, p. 22; FARRAN, 1936, p. 77; MORI, 1937, p. 17, pl. 5; *Nannocalanus minor*, VERVOORT, 1947, p. 26.

This species of *Calanus* has been transferred to the genus *Nannocalanus* by G. O. SARS by the fact that the head is fused with the 1st thoracic segment, and by the structure of male 4th pair of legs which differ from those of the typical *Calanus*. SEWELL recorded two forms, differing each other only in size, from the Indian waters.

Length. Female, 1.77–1.84 mm. Male, 1.50–1.56 mm.

Occurrence. The species is not so common in the Izu region. A vertical haul from 1000 m to the surface in Suruga Bay contained three adult females.

Distribution. The species has a wide distribution in the tropical and subtropical regions of the Pacific, Atlantic and Indian Oceans. In Japan it has been recorded from warm currents.

Genus *Calanoides* BRADY, 1883

The genus was separated from the typical *Calanus* by the possession of prehensile structure in the male 5th legs, and in having 4 marginal setae in the terminal joint of the endopodite of 5th leg of the female. The genus is represented by three species, *Calanoides carinatus* KRÖYER (=*brev.* LUBBOCK), C. *pathagoniensis* BRADY and *C. acutus* GIESBRECHT. One of them occurred in the present collection.

Calanoides carinatus KRÖYER

Calanus brevicornis GIESBRECHT, 1892, p. 90, t. 6, 7, 8; *Calanoides brevicornis* A. SCOTT, 1909, p. 10; TANAKA, 1937, p. 251, fig. 1; *C. carinatus* VERVOORT, 1947, p. 29, figs. 1–3.

The specimen collected was an immature female, measuring 2.75 mm. The species has been recorded from the Malayan region by A. SCOTT. CLEVE recorded it from the Indian Ocean but SEWELL failed to detect it in the "Investigator" collection.

Genus *Canthocalanus* A. SCOTT, 1909

This genus was created by A. SCOTT to accomodate a species which differs from the typical *Calanus* in the peculiar structure of the 2nd basal joint of the 1st leg, and the characters of the male 5th legs. The hook is observed on the 2nd basal joint of the 1st leg of the following four species: *Canthocalanus pauper* (GIESBRECHT), *Calanus robustior* (GIESBRECHT), *Calanus gracilis* (DANA) and *Megacalanus princeps* (WOLFENDEN). According to A. SCOTT, the hook on the 2nd basal joint of the 1st leg of *Canthocalanus* is quite different from that of the other three species, *viz.*: the

2nd joint is furnished with a strong, naked pyriform spine on its anterior surface; the base of this spine is only slightly attached to the surface of the joint; a considerable portion of the spines is continued upward into a distinct tooth-like process, resulting with the combination of the hook on the 1st joint, in the formation of a feeble prehensile apparatus. Further he states on the hook which is found in the 2nd basal of the 1st legs of *Megacalanus*: "This hook is quite distinct from that found in *Canthocalanus*. In *Megacalanus* the hook is a well-defined projection from the surface of the joint, and has a plumose seta articulated to it. In *Canthocalanus* the seta is transformed into a strong spine with a hooked base." A. SCOTT further emphasizes as a characteristic of *Canthocalanus* on the naked rudimentary 3-jointed endopodite of the left 5th leg of the male but this rudimentary endopodite is observed also in the left 5th leg of *Calanus robustior* GIESBRECHT. Were it sufficient reasons proposed by A. SCOTT to separate the genus *Canthocalanus* from the typical *Calanus* that the species of *Canthocalanus* has a hook and a strong spine on the 2nd basal of the 1st leg of the female, and naked endopodite on the left 5th leg of the male, one could have well transferred *Calanus robustior* into the genus *Canthocalanus*. A. SCOTT, however, included *Calanus robustior* and *C. gracilis* in the genus *Megacalanus* only on the ground that these three forms have a similar hook on the 2nd basal of the 1st leg. But he does not care the fact that in the female of *Calanus robustior* and *C. gracilis*, the head and the 1st thoracic segment are fused, and the distal spine of the terminal joint of the exopodite of the 1st leg is of peculiar shape, whereas in *Megacalanus* the head and the 1st thoracic segment are separate. Moreover, in *Megacalanus*, the distal joint of the exopodite of 2nd to 4th leg has three outer marginal spines, and the terminal spine of the 3rd joint of the exopodite of the 1st leg is of usual structure.

It seems unreasonable, as cited above, that A. SCOTT has included *C. robustior* and *C. gracilis* in the genus *Megacalanus*. They should be transferred to other genus. It is advisable to keep them in the genus *Neocalanus* proposed by G. O. SARS.

Canthocalanus pauper (GIESBRECHT)

Calanus pauper GIESBRECHT, 1892, p. 91, t. 6, 8; *Canthocalanus pauper* A. SCOTT, 1909, p. 9; *Calanus pauper* FARRAN, 1929, p. 215; *Canthocalanus pauper* SEWELL, 1929, p. 25; *Calanus pauper* FARRAN, 1936, p. 77; MORI, 1937, p. 18, pl. 6; *Canthocalanus pauper*, VERVOORT, 1947, p. 36.

Length. Female, 1.48–1.62 mm. Male, 1.40 mm.

Occurrence. The species is rare in the Izu region. Only two adult females and one male have been collected from the surface layer.

Distribution. The species has been recorded from the tropical and subtropical regions of the Pacific and Indian Oceans. In Japanese waters it has been recorded from the warm currents.

Genus *Neocalanus* G. O. Sars, 1925

Calanus robustior Giesbrecht and *C. gracilis* Dana have been often transferred from one genus to another by different authors. A. Scott accomodated them in the genus *Megacalanus* Wolfenden on the ground that they have strong hook on the 2nd basal joint of the 1st leg. But he does not mention the absence of the peculiar terminal spine on the exopodite of the 1st leg. Sars separated these two species from the genus *Megacalanus* by the fact that in these two species the head and the 1st thoracic segment are fused, and by the features of the male 5th legs. He included these species along with *C. tenuicornis* Dana in his proposed genus *Neocalanus* without paying any attention to the presence of a strong hook on the 2nd basal joint of the 1st leg in *C. robustior* and *C. gracilis*. If it were Sars' main reason for including *C. tenuicornis* in the genus *Neocalanus* that it has the similar structure in the male 5th pair of legs to those of *robustior* and *gracilis* we had better accomodate *C. robustior* in the genus *Canthocalanus* and *A. gracilis* in the typical *Calanus*. However, the female specimen of *robustior* and *gracilis* have the similar structure in the 1st leg, and the same number of segments in the cephalothorax which differ from that of *C. tenuicornis*. It is advisable to remove *C. tenuicornis* from the genus *Neocalanus*, and keep it in the typical *Calanus*.

Neocalanus gracilis (Dana)

Calanus gracilis Giesbrecht, 1892, p. 90, t. 1, 6, 7, 8; *Megacalanus gracilis*, A. Scott, 1909, p. 12; *Neocalanus gracilis* G. O. Sars, 1925, p. 7; *Calanus gracilis* Farran, 1929, p. 217; *Neocalanus gracilis* Sewell, 1929, p. 26; Wilson, 1932, p. 28; *Calanus gracilis* Mori, 1937, p. 16, pl. 3, 4; *Neocalanus gracilis*, Vervoort, 1947, p. 40.

Length. Female, 2.95–3.25 mm.

Remarks. The male of this species has the 1st thoracic segment separated from the head, and the 5th pair of legs which resemble of *C. tenuicornis* Dana, but the 1st basal joint of the left 5th leg is longer than that of the right, and the exopodite of the left leg much elongated.

Occurrence. The species is rather rare in the Izu region. It was collected by the vertical hauls from 1000 m to the surface in Sagami and Suruga Bay.

Distribution. The species has been recorded from the tropical and subtropical regions of the Pacific, Atlantic and Indian Oceans.

Neocalanus robustior (Giesbrecht)

Calanus robustior Giesbrecht, 1892, p. 91, t. 7, 8; Esterly, 129; *Megacalanus robustior* A. Scott, 1909, p. 13; *Neocalanus robustior* G. O. Sars, 1925, p. 8; *Calanus robustior* Farran, 1929, p. 217; Mori, 1937, p. 17, pl. 3, 4; *Neocalanus robustior*, Vervoort, 1947, p. 44.

Length. Male, 3.00 mm.

Remarks. The male specimen has the 1st thoracic segment which is separated from the head. The left 5th leg of the male resembles that of *Canthocalanus pauper* in having a rudimentary 3-jointed endopodite.

Occurrence. The species is very rare in the Izu region. A single male specimen was obtained in a surface haul at Misaki in winter of 1930.

Distribution. The species has been recorded from the tropical and subtropical regions of the Pacific, Atlantic and Malay Archipelago. In Japan, it has been recorded from the warm currents.

Genus *Megacalanus* WOLFENDEN, 1904

The genus was established by WOLFENDEN in 1904 to accomodate a large copepod which he obtained from the deep water of the Atlantic, and called it *Megacalanus princeps*. The genus is distinguished from the typical *Calanus* in having a remarkable hook on the anterior surface of the 2nd basal joint of the 1st leg, and the presence of three outer marginal spines on the 3rd joint of the exopodite of the 2nd to 4th legs. In the same year SARS described the same species under the name *Macrocalanus longicornis*. WOLFENDEN (1905) added a second species of *Megacalanus* which he considered to be identical with *Calanus princeps* BRADY. So the specific name of the first form, *M. princeps*, was renamed *M. bradyi* and the name *M. princeps* was applied to the second form which is clearly not *Megacalanus*. A. SCOTT (1909) created a new genus *Bradycalanus*, and suggested that *Calanus princeps* BRADY may probably belong to his proposed genus. SARS, however, included *Bradycalanus* into *Megacalanus* on the ground that these genera differ only in the presence or absence of a hook on the 2nd basal joint of the 1st leg. The genus *Megacalanus* proposed by SARS is, at present, generally accepted, and the genus comprises three species: *M. princeps* WOLFENDEN, *M. sarsi* FARRAN, which is identical with *Macrocalanus princeps* SARS 1905, and *M. typicus* (A. SCOTT). One of them appeared in the collection.

Megacalanus princeps WOLFENDEN

(Fig. 3, *a–d*)

Megacalanus princeps A. SCOTT, 1909, p. 13, pl. 1; *M. longicornis* SARS, 1925, p. 14, pl. 3; SEWELL, 1929, p. 27; *M. princeps* VERVOORT, 1947, p. 49, fig. 4.

Female. Length, 10.34 mm; cephalothorax, 18.15, abdomen, 2.19 mm; the cephalothorax elongate ovate; the lateral corners of the last thoracic segment triangularly produced; the rostrum consists of two strong spines.

The abdomen 4-segmented; the segments and furca in the proportions 41 : 20 : 15 : 7 : 17 = 100; the furcal rami slightly longer than wide.

The 1st antenna 25-jointed, exceeds the end of the furca by terminal nine joints. The mouth parts as those of the genus *Calanus*. The maxillipede is furnished with stiff hairs on the middle part of the 1st basal, and on the proximal half of the 2nd basal joint.

Fig. 3. *Megacalanus princeps* WOLFENDEN.
a, Female, dorsal aspect; *b*, head, lateral aspect; *c*, last thoracic segment and abdomen, lateral aspect; *d*, basal joints and endopodite of 1st leg.

The 1st to 5th legs have each 3-jointed exopodite and endopodite. There is a prominent hook on the 2nd basal joint of the 1st leg at the junction with the endopodite. The outer margin of the 3rd joint of the exopodite of the 2nd to 4th legs has

three spines. The 5th leg has two outer marginal spines on the 3rd joint of the exopodite.

Remarks. *Pseudolovenula magna* MARUKAWA is, according to VERVOORT, identical with the present species.

Occurrence. Five female specimens from the deep waters of Sagami and Suruga Bay.

Distribution. The species has wide distribution in the deep waters of the Pacific, Atlantic, and Indian Oceans.

Genus *Bradycalanus* A. SCOTT, 1909

A. SCOTT created the genus *Bradycalanus* to accomodate the species which resembles closely *Megacalanus* WOLFENDEN. His genus is distinguished from the latter by the densely plumose spine on the apex of the 2nd maxilla, and by the absence of the hook on the anterior surface of the 2nd basal joint of the 1st leg. The other appendages are as those in *Megacalanus*. SARS merged the genus *Bradycalanus* into *Megacalanus* in his monograph of 1925. The genus *Bradycalanus* is represented by a single species *B. typicus* A. SCOTT. VERVOORT recorded the species in the Snellius Expedition under the name *Megacalanus typicus*. No example of the genus has been met with in the present collection.

Genus *Bathycalanus* G. O. SARS, 1905

The genus was created by SARS to accomodate a single species, *B. richardi*, which was obtained from the Atlantic. The genus *Bathycalanus* differs from *Megacalanus* WOLFENDEN in the peculiar armature of the frontal part of the head, and in the structure of the maxillae and maxillipede, and the 1st pair of legs. The 1st leg has no hooked process on the 2nd basal joint, and the exopodite is composed of two joints.

At present three species, *B. richardi* SARS, *B. bradyi* WOLFENDEN (=*Megacalanus bradyi* WOLFENDEN, 1905) and *B. princeps* (BRADY) (=*Calanus princeps* BRADY, 1883) have been reported. SEWELL recorded *B. richardi* SARS from the Indian Seas. VERVOORT recorded three species in the Snellius Expedition. But no example of the genus has been met with in the Izu region.

Genus *Undinula* A. SCOTT, 1909

The genus was named by A. SCOTT to accomodate some species of *Calanus* which exhibit a considerable difference in the structure of the male 5th pair of legs. The female of the genus differs very little in the structure of the appendages from typical *Calanus*. The genus, at present, comprises two species, *U. darwini* (LUBBOCK) and *U. vulgaris* (DANA). Both of them appeared commonly in the Izu region.

Undinula darwini (LUBBOCK)

Calanus darwini GIESBRECHT, 1892, p. 91, t. 6, 7, 8; *Undinula darwini* A. SCOTT, 1909, p. 17; FARRAN, 1929, p. 217; SEWELL, 1929, p. 42; FARRAN, 1936, p. 78; *Calanus darwini*, MORI, 1937, p. 18, pl. 5; *Undinula darwini*, VERVOORT, 1947, p. 77.

Length. Female, 2.13–2.56 mm. Male, 1.93–2.1 mm.

Remarks. SEWELL divided the female specimen into three forms: forma *typica*, forma *typica* var. *symmetrica*, and forma *typica* var. *intermedia*; the present specimen is identical with forma *typica*, but the posterior margin of the thoracic segment is not produced on the right side as figured by SEWELL.

Occurrence. The species is one of the most common surface-living one in the Izu region.

Distribution. The species is widely distributed in the warm waters of the Pacific, Atlantic and Indian Oceans.

Undinula vulgaris (DANA)

Calanus vulgaris GIESBRECHT, 1892, p. 92, t. 6, 7, 8; *Undinula vulgaris* A. SCOTT, 1909, p. 18; *Calanus vulgaris* FARRAN, 1929, p. 216; *Undinula vulgaris* SEWELL, 1929, p. 81; WILSON, 1932, p. 29; FARRAN, 1936, p. 77; *Calanus vulgaris* MORI, 1937, p. 19, pl. 5, 6; *Undinula vulgaris* VERVOORT, 1947, p. 72.

Length. Female, 2.75–2.81 mm. Male, 2.31–2.69 mm.

Remarks. MORI (1937) recorded an abnormal specimen with branched furcal setae which had previously been described as *Calanus orientalis* by MARUKAWA (1908).

Occurrence. The species is one of the most common surface-living copepod of the Izu region; it is abundant in summer season.

Distribution. The species has a wide distribution in the warm regions of the oceans.

Family EUCALANIDAE

Genus *Eucalanus* DANA, 1848

Eight species belonging to the genus have been recorded from the Japanese waters, namely: *E. attenuatus* (DANA), *E. elongatus* (DANA), *E. crassus* GIESBRECHT, *E. mucronatus* GIESBRECHT, *E. pileatus* GIESBRECHT, *E. subcrassus* GIESBRECHT, *E. subtenuis* GIESBRECHT and *E. giesbrechti* MORI; all of them occurred in the present collection. Beside these, *E. bungii californicus* JOHNSON was found from the deep water of Suruga Bay. It has been reported by GIESBRECHT that there are three varieties of *Eucalanus elongatus* DANA: var. *hyalinus*, var. *inermis* and var. *bungii*; of these, *hyalinus* is the Atlantic form. According to Dr. M. W. JOHNSON var. *hyalinus* and var. *inermis* are the tropical or sub-tropical variant and var. *bungii* is the boreal variant of the Pacific. Of these three varieties, var. *bungii* has been obtained abundantly in the North-East

Pacific; var. *inermis* and var. *hyalinus* in small number off the coast of California. He, after studying the developmental stages and the geographical distribution of these varieties, raised var. *bungii* and var. *inermis* to specific rank. Moreover, he established a new subspecies of var. *bungii* which is the southern form of var. *bungii*, and named *E. bungii californicus*. Of these variants, I have recorded the occurrence of var. *bungii* from the surface water of Sagami Bay in winter season, but the occurrence of var. *bungii californicus* has not been known untill the deep water hauls in Suruga Bay caught it in the collection. Nine species, in all, have been obtained in the present material; they appear to be the warm water inhabitant except *E. bungii bungii* and *E. bungii californicus*.

According to SEWELL, *Eucalanus acus* FARRAN, taken in the south of New Zealand, is synonym of *E. longiceps* MATHEWS which had been collected in the Indian Seas. SEWELL recorded the occurrence of eight species from the Indian Seas, namely, *E. attenuatus*, *E. elongatus*, *E. crassus*, *E. longiceps*, *E. monachs*, *E. mucronatus*, *E. pileatus*, and *E. subcrassus*. A. SCOTT recorded also six species with the exception of *E. attenuatus* and *E. elongatus*. VERVOORT recorded seven species in the Snellius Expedition. He is of opinion that JOHNSON's species of *E. elongatus* has no specific value.

Eucalanus attenuatus (DANA)

(Fig. 4, *a–d*)

Eucalanus attenuatus GIESBRECHT, 1892, p. 131, t. 3, 11, 35; ESTERLY, 1905, p. 133; G. O. SARS, 1925, p. 78; FARRAN, 1929, p. 218; SEWELL, 1929, p. 47; WILSON, 1932, p. 32; TANAKA, 1935, p. 145, pl. 11; MORI, 1937, p. 22, pl. 8; FARRAN, 1936, p. 78; VERVOORT, 1947, p. 95.

Length. Female, 4.32–4.43 mm, Male, 3.24–3.42 mm.

Occurrence. Very common in the surface and deep layers.

Distribution. The species is widely distributed in the Pacific, Atlantic and Indian Oceans.

Remarks. Among the specimens of *E. attenuatus* DANA taken in winter season from Sagami Bay, there occurred often large individuals which differ slightly from the typical *E. attenuatus* in the proportional lengths of the joints of the male 5th pair of legs (fig. 4 b). The specimen measured 4.76 mm in the female, and 4.40–4.54 mm in the male.

There is another form of *E. attenuatus* which differs either of them above stated in the following: the genital segment of the female is wider than long (fig. 4 c); the thoracic segments and the 1st to 2nd abdominal segments are covered with fine short hairs; this specimen measured 5.72–6.19 mm in the female, and 4.50–4.67 mm in the male of 5th copepodid stage (fig. 4 d, e).

These specimens were taken mixed with the typical form of *E. attenuatus* in winter 1930, from the surface water of Sagami Bay; they may perhaps be the nor-

thern varieties of *E. attenuatus* (DANA).

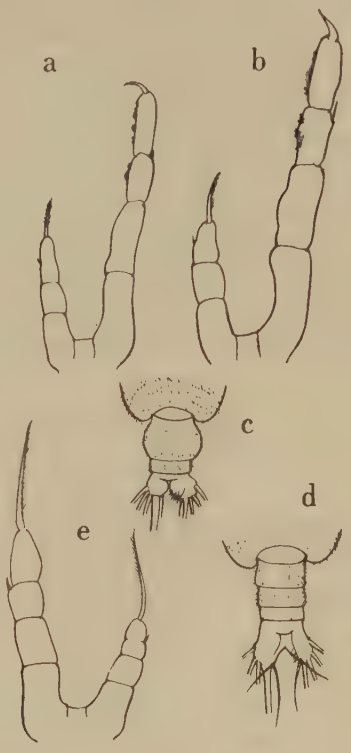

Fig. 4. *Eucalanus attenuatus* (DANA). *a*, Male: 5th pair of legs of the type specimen; *b*, 5th pair of legs of large specimen, measuring 4.40–4.54 mm; *c*, female: abdomen of large specimen; *d*, male: juv. larger specimen, abdomen, dorsal aspect; *e*, 5th pair of legs.

Eucalanus elongatus (DANA)

Eucalanus elongatus GIESBRECHT, 1892, p. 131, t. 11, 35; G. O. SARS, 1925, p. 20; FARRAN, 1929, p. 218; SEWELL, 1929, p. 48; WILSON, 1932, p. 31; TANAKA, 1935, p. 143, pl. 1; FARRAN, 1936, p. 78; MORI, 1937, p. 21, pl. 7; VERVOORT, 1947, p. 84.

Length. Female, 5.62–5.76 mm. The specimen has laterally produced thoracic margins, and is slightly spinulated on the dorsal surface. The 1st leg has an internal setae on the 2nd basal joint.

Occurrence. The species has been obtained both from the surface and deep waters in small quantity.

Distribution. The species has wide distribution in the Pacific, Atlantic and Indian Oceans.

Eucalanus bungii var. *bungii* (JOHNSON)

Eucalanus elongatus var. *bungii* GIESBRECHT, 1892, p. 149; SATO, 1913, p. 2; *Eucalanus elongatus* var. *bungii* TANAKA, 1935, p. 143, pl. 1; *Eucalanus giesbrechti* MORI, 1937, p. 22, pl. 7; *Eucalanus bungii bungii* JOHNSON, 1938, p. 167, figs. 4-10, 18, 20, 23, 25; *Eucalanus elongatus* VERVOORT, 1947, p. 84.

Length. Female, 6.30 mm. Male, copepodid stage V, 5.50 mm.

Remarks. This species is easily distinguished from *E. elongatus* by the rounded corners of the 5th thoracic segment, and by the presence of three setae on the 2nd basal joint of the mandible. MORI raised the species to the rank of species under the name *E. giesbrechti*. JOHNSON revised it as *E. bungii bungii*, a subspecies of *E. bungii*. VERVOORT advices to distinguish two forms in *E. elongatus*: the Pacific form with smoothly rounded lateral thoracic margins, symmetrical 1st antennae and without any indication of spinules on the dorsal surface and, the Atlantic form with produced thoracic margins, asymmetrical 1st antenna and spinulated dorsal surface. But in my opinion it is better to distinguish the former with rounded thoracic margins from the latter with produced thoracic margins under the name *E. bungii*. The present specimen, *E. bungii* var. *bungii*, is the northern variety of *bungii*.

Eucalanus bungii var. *californicus* (JOHNSON)

(Fig. 5, *a–d*)

Eucalanus elongatus ESTERLY, 1905, p. 131.
Eucalanus bungii californicus JOHNSON, 1938, p. 169, fig. 22.

Remarks. This species agrees with *E. elongatus* var. *bungii* GIESBRECHT except that the 2nd basal of the mandible is furnished with a single seta. According to JOHNSON, *E. bungii* var. *bungii* is a northern variety of *E. elongatus*, and *E. bungii californicus* is a southern variety of *E. bungii* which is common in the California coast. According to JOHNSON, *E. elongatus* ESTERLY is identical with the present species. JOHNSON elevated this species to the rank of subspecies, but it seems reasonable to keep the subspecies as a variety.

Length. Female, 6.18 mm, cephalothorax, 5.62 mm, abdomen, 0.56 mm. The cephalothorax elongate oval, produced narrowly anteriorly. The abdomen is contained 10-times in the length of the cephalothorax; the abdomen 3-segmented, the segments and the furca in the proportions 47 : 18 : 12 : 23 = 100; the furcal rami slightly asymmetrical.

The 1st antenna exceeds the end of the furca by terminal two or three joints. The 2nd antenna has 8-jointed endopodite; the 1st and 2nd basal joints of the exopodite are of equal lengths. The mandible has a slender 2nd basal joint which is furnished with one seta on the proximal inner margin; the exopodite is situated about the middle of the 2nd basal joint. The maxillae and maxillipede are as those of *E. elongatus* (DANA).

The 3rd joint of the exopodite of 1st leg has two outer marginal spines. The 2nd to 4th legs as those of *E. elongatus*.

Occurrence. The species has been collected, only in small numbers, from the deep water of Suruga Bay, but not a single specimen from Sagami Bay.

Distribution. The specimen has been recorded only from the California coast.

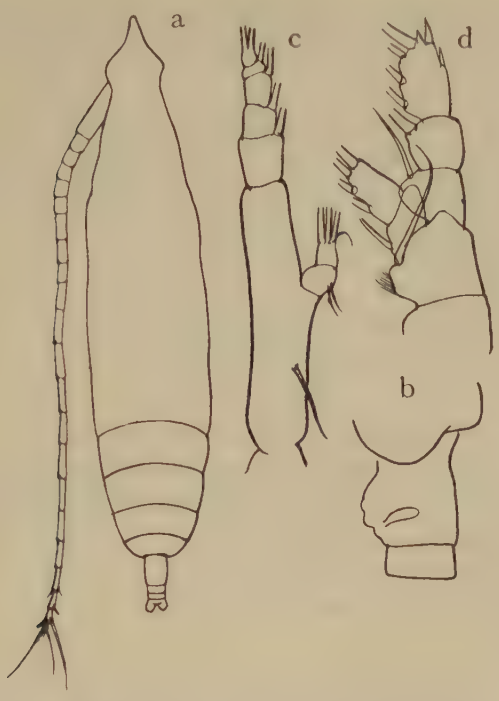

Fig. 5. *Eucalanus bungii* var. *californicus* (JOHNSON).
a, Female, dorsal aspect; *b*, last thoracic segment and genital segment, lateral aspect; *c*, mandible; *d*, 1st leg.

Eucalanus crassus GIESBRECHT

Eucalanus crassus GIESBRECHT, 1892, p. 132, t. 11, 35; ESTERLY, 1905, p. 134; A. SCOTT, 1909, p. 19; WITH, 1915, p. 53; SARS, 1925, p. 22; FARRAN, 1929, p. 220; SEWELL, 1929, p. 50; FARRAN, 1936, p. 78; TANAKA, 1935, p. 145, pl. 5; MORI, 1937, p. 23, pl. 9; VERVOORT, 1947, p. 112; *E. oculanus* MARUKAWA, 1921, p. 7.

Length. Female, 3.17–3.73 mm. Male, 3.13–3.49 mm.

Occurrence. Common in the surface water.

Distribution. The species is widely distributed in the warm regions of the oceans.

Eucalanus mucronatus GIESBRECHT

Eucalanus mucronatus GIESBRECHT, 1892, p. 132, t. 11, 35; A. SCOTT, 1909, p. 20; G. O. SARS, 1925, p. 21; FARRAN, 1925, p. 218; TANAKA, 1935, p. 147, pl. 3; FARRAN, 1936, p. 78; MORI, 1937, p. 25, pl. 8; VERVOORT, 1947, p. 104.

Length. Female, 3.13–3.49 mm.

Occurrence. Rather rare; the male specimens were all immature.

Distribution. The species has a wide distribution in the warm regions of the oceans, but according to SEWELL the species is not common in the Indian Ocean.

Eucalanus pileatus GIESBRECHT

Eucalanus pileatus GIESBRECHT, 1892, p. 132, t. 11, 35; A. SCOTT, 1909, p. 21; WOLFENDEN, 1911, p. 204; SEWELL, 1929, p. 51; TANAKA, 1935, p. 148, pl. 4.

Length. Female, 2.11–2.41 mm. Male, 2.16 mm.

Occurrence. Rather rare.

Distribution. The species has been recorded from the Pacific, Atlantic, Malay Archipelago, Maldive Archipelago and Indian Ocean.

Eucalanus subcrassus GIESBRECHT

Eucalanus subcrassus GIESBRECHT, 1892, p. 132, pl. t. 11, 35; A. SCOTT, 1909, p. 21; FARRAN, 1929, p. 219; SEWELL, 1929, p. 51; TANAKA, 1935, p. 51, pl. 4; FARRAN, 1936, p. 75; MORI, 1937, p. 24, pl. 9; VERVOORT, 1947, p. 108.

Length. Female, 2.77–2.82 mm. Male, 2.52 mm.

Occurrence. Rare.

Distribution. The species is widely distributed in the tropical and subtropical regions, and has been recorded from the Atlantic, Indian Oceans, Malay Archipelago and Great Barrier Reef.

Eucalanus subtenuis GIESBRECHT

Eucalanus subtenuis GIESBRECHT, 1892, p. 132, t. 11, 35; ESTERLY, 1905, p. 135; MARUKAWA, 1908, p. 6, pl. 1, 2; A. SCOTT, 1909, p. 21; G. O. SARS, 1925, p. 21; FARRAN, 1929, p. 218; TANAKA, p. 147, pl. 3; MORI, 1937, p. 25; VERVOORT, 1947, p. 106.

Length. Female, 3.22–2.40 mm. Male, 3.06–2.63 mm.

Occurrence. One of the most common species.

Distribution. The species has been recorded from the Pacific, Atlantic, Mediterranean Sea, and Malay Archipelago; but the occurrence of the species has not been recorded from Indian Ocean.

Genus *Rhincalanus*, DANA, 1848

Three species belonging to this genus have been recorded from the Japanese waters: *Rhincalanus cornutus* DANA, *R. nasutus* GIESBRECHT and *R. gigas* BRADY. The last species has been described by MARUKAWA (1908) from the immature specimen taken from the surface waters off the coast of the Prov. Izu. His figures are, as pointed out by SCHMAUSS, uncertain. He described that the specimen is a female, measuring 7.8–9.0 mm in total length; but the figure (Fig. 59) given as the 5th pair of legs,

indicates that the specimen is not female at all, but an immature male; the 2nd to 4th thoracic segments have each a pair of dorsal and lateral spines and dorsal spines on the 1st abdominal segment. The immature male specimen of *R. gigas* has the lateral spines on the 3rd and 4th thoracic segment, and dorsal spines only on the 4th thoracic and on the 1st abdominal segment. MARUKAWA's figure of the 5th pair of legs agrees those of *R. gigas* or *R. nasutus*, in having a long endopodite on the left leg. His specimen differs from *R. gigas* in the spinulation of the thoracic segments which is the sufficient reason for identifying the both species. His specimen measures about 3.2-5.4 mm, calculating from the magnification of the microscope used, and from the figures of the entire body of the other species on the same plate. Again considering the geographical distribution of *R. gigas*, it is confined to the Antarctic water south of Lat. 37° S; the occurrence of the species in the upper layer of the northern temperate region seems very doubtful. SCHMAUSS identified MARUKAWA's specimen as *R. cornutus* forma *typica*; perhaps for the reasons that the specimen has both lateral and dorsal spines on the 2nd thoracic segment; but the shape of the forehead quite differs from that of *R. cornutus* in MARUKAWA's specimen. *R. nasutus* in Japanese waters has both lateral and dorsal spine on the 2nd thoracic segment. From the reasons above stated, MARUKAWA's specimen is the immature male of *R. nasutus* which is common in Japanese waters.

Rhincalanus nasutus GIESBRECHT

Rhincalanus nasutus GIESBRECHT, 1892, p. 132, t. 3, 9, 12, 35; G. O. SARS, 1903, p. 18; ESTERLY, 1905, p. 136; *R. gigas* MARUKAWA, 1908, p. 3, pl. 2, figs. 49-59; A SCOTT, 1909, p. 24; SCHMAUSS, 1927, p. 368; FARRAN, 1929, p. 220; SEWELL, 1929, p. 58; WILSON, 1932, p. 34; TANAKA, 1935, p. 151; FARRAN, 1936, p. 79; MORI, 1937, p. 26, pl. 10; VERVOORT, 1949, p. 122.

Length. Female, 3.60–4.50 mm. Male, 3.74–3.81 mm.

Remarks. The specimen from the Japanese waters has both lateral and dorsal spines on the 2nd thoracic segment.

Occurrence. Common. It is rather abundant in the deep layers.

Distribution. The species has a wide distribution in the oceans.

Rhincalanus cornutus DANA

Rhincalanus cornutus GIESBRECHT, 1892, p. 153, t. 12; A. SCOTT, 1909, p. 32; G. O. SARS, 1925, p. 22; SCHMAUSS, 1927, p. 359; FARRAN, 1929, p. 220; SEWELL, 1929, p. 58; WILSON, 1934, p. 35; TANAKA, 1935, p. 151; FARRAN, 1936, p. 79; MORI, 1937, p. 27, pl. 10; VERVOORT, 1947, p. 116.

Length. Female, 3.24–3.63 mm.

Remarks. The specimen agrees well with description and figures of *R. cornutus* f. *typica* given by SCHMAUSS. The male specimens were all immature in the 5th copepodid stage.

Occurrence. Common in the surface layer.

Distribution. The species has a wide distribution in the oceans.

Genus *Mecynocera* THOMPSON, 1888

Mecynocera clausi THOMPSON

Mecynocera clausi GIESBRECHT, 1892, p. 160, t. 5, 11, 35; THOMPSON, 1894, p. 80; ESTERLY, 1905, p. 137; SARS, 1925, p. 23; FARRAN, 1929, p. 221; SEWELL, 1929, p. 60; WILSON, 1932, p. 36; TANAKA, 1935, p. 152, p. 4; FARRAN, 1936, p. 79; MORI, 1937, p. 28, pl. 11, 12.

Female. Length, 0.94–0.99 mm.

Occurrence. Common species in the surface layer.

Remarks. The adult male specimen has not been met with in Japanese waters. It has been recorded from the Gulf of Guinea. The male 5th legs have been described as follows: "The 5th pair are one-branched, alike on both sides and in both sexes; basal portion two-jointed; not much stouter than the terminal portion which is three-jointed. There is, so far as could be observed, no terminal spine."

Distribution. The species is of a wide distribution, and has been recorded from warm waters of the oceans.

THE PELAGIC COPEPODS OF THE IZU REGION, MIDDLE JAPAN
SYSTEMATIC ACCOUNT II.
FAMILIES PARACALANIDAE AND PSEUDOCALANIDAE

Otohiko TANAKA

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THE PELAGIC COPEPODS OF THE IZU REGION, MIDDLE JAPAN SYSTEMATIC ACCOUNT II.

FAMILIES PARACALANIDAE AND PSEUDOCALANIDAE

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With 17 Text-figures

Family PARACALANIDAE

Genus *Paracalanus* Boeck, 1884

Three species belonging to this genus have been collected from the Izu region: *Paracalanus aculeatus* Giesbrecht, *P. parvus* (Claus) and *P. denudatus* Sewell. It has been reported by many authors that some of the species have two forms differing from each other mainly in size. The specimens belonging to this genus are, generally, of small size, and structually alike; one cannot get a correct taxomonic determination of the species, unless he observes to every minute point of structure. Moreover, there happens, often, some variations in the armature and structure of the swimming legs, especially, in the 5th legs of the female. From the Japanese waters, Mori recorded the occurrence of *P. aculeatus* and *P. parvus*. Sewell recorded from the Indian seas the occurrence of *P. aculeatus*, *P. serratipes* Sewell, *P. denudatus* Sewell, *P. parvus* (Claus), *P. nannus* Sars, *P. crassirostris* Dahl, *P. dubia* Sewell and *P. nudus* Sewell; he arranged them into two groups, namely, the *aculeatus* and the *parvus* groups; the former three belong to the *aculeatus* and the latter five to the *parvus* group.

Paracalanus aculeatus Giesbrecht

Paracalanus aculeatus Giesbrecht, 1892, p. 146, t. 9; Wolfenden, 1909, p. 998; A. Scott, 1909, p. 26; Sewell, 1914, p. 204; Farran, 1929, p. 222; Sewell, 1929, p. 62; Farran, 1936, p. 79; Mori, 1937, p. 30, pl. 11; Vervoort, 1947, p. 127.

Length. Female; 1.18–1.36 mm. Male; 1.27–1.36 mm.

Remarks. The present female specimen, though much larger in size than those described by Sewell as *P. aculeatus* forma *major* taken from the Indian sea, agrees

fairly well with his description. The lengths of the terminal spine on the 3rd joint of the exopodite of the 2nd and 3rd legs appear somewhat shorter, when compared with SEWELL's figures. A transversal row of the spinules on the distal margin of the 5th legs is much reduced, and only a single spinule was observed near the inner distal margin; in the SEWELL's figure, the spinule on the distal margin is three in number. In the male the proportional lengths of the joint of the left 5th leg differ from those given by SEWELL (1914, p. 208, 15:15:15:12); the 4th joint is the longest in my specimen (20:13:21:25:21=100).

Occurrence. The species is fairly common, but not abundant.

Distribution. The species appears to be distributed in the tropical and subtropical regions of the oceans. In Japan it has been recorded from the warm regions.

Paracalanus denudatus SEWELL

(Fig. 6, *a–b*)

Paracalanus denudatus SEWELL, 1929, p. 66, fig. 23; FARRAN, 1936, p. 80, fig. 1.

Female. Length, 0.74–0.81 mm. The specimen closely resembles *P. aculeatus* forma *major*. The dorsal profile exhibits a slight hump about the middle of the fused mass of the head and the 1st thoracic segment. The abdomen is contained 3.6–times in the length of the cephalothorax. The abdomen 4–segmented; the segments and furca are in the proportional lengths 35:10:9:24:22=100; the genital segment wider than long; the genital orifice as that of *P. aculeatus* forma *minor*; the furcal rami about 2–times as long as wide, and set close together.

The 1st antenna exceeds the end of the abdomen by terminal two joints; the distal joint is 1.6–times as long as the penultimate one; the joints 8 and 9 are completely separate; the joints 10 to 22 are furnished with rows of minute spinules on the posterior margin.

In the 1st leg the 1st joint of the exopodite is furnished with a minute outer edge spine. In the 2nd to 4th legs the spinulation on the posterior surface of the 2nd joint of the endopodite, and the outer marginal teeth on the 3rd joint of the exopodite are much reduced; the number of spines on these joints, and the proportional lengths of the 3rd joint of the exopodite to the end spine are as follow:

	Present specimen				SEWELL'S specimen				FARRAN'S specimen	
	Sp. Ri 2	Sp. Re 3	Re 3	Spt.	Sp. Ri 2	Sp. Re 3	Re 3	Spt.	Re 3	Spt.
2nd	2–3	5–6	100	138	2	5	100	136	100	128
3rd	3–5	8–11	100	118	3	8	100	126	100	104
4th	4–5	7–8	100	109	4	4	100	103	100	85

The 5th pair of legs are small, and resemble those of *P. aculeatus* forma *minor*; the 1st joint about as long as the 2nd; the 2nd joint about 3-times as long as wide, ending in two unequal terminal spines. The other specimen, measuring 0.8 mm, had 3-jointed 5th legs which resemble those of *P. denudatus* figured by SEWELL (fig. 23, *h*); another specimen (0.74 mm) had an abnormal 5th legs as those figured by SEWELL as the 5th legs of *P. aculeatus* forma *minor*.

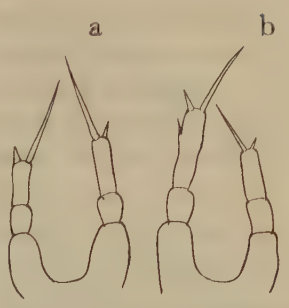

Fig. 6. *Paracalauns denudatus* SEWELL.
a, *b*, Female: abnormal 5th legs.

Remarks. The present specimen, though larger in size than those reported by SEWELL, agrees more closely to *P. denudatus* than to *P. aculeatus* forma *minor* SEWELL in having a long furcal rami, the armature of the 3rd joint of the exopodite, and the proportional lengths of the 3rd joint of the exopodite to the endspine of the 2nd to 4th legs. The 5th pair of legs differ from the type figured by SEWELL, but according to FARRAN, the 5th legs are 3-jointed, and sometimes 2-jointed.

Occurrence. The species has been obtained from the surface; it is not common here.

Distribution. The species has been recorded from the Indian seas and Great Barrier Reef.

Paracalanus parvus (CLAUS)

Paracalanus parvus GIESBRECHT, 1892, p. 164, t. 1, 6, 9; G. O. SARS, 1903, p. 17; ESTERLY, 1905, p. 140; WOLFENDEN, 1906, p. 998; A. SCOTT, 1909, p. 27; SATO, 1903, p. 15; SEWELL, 1914, p. 208; WITH, 1915, p. 54; ESTERLY, 1924, p. 86; G. O. SARS, 1925, p. 24; FARRAN, 1926, p. 233; GURNEY, 1927, p. 143; FARRAN, 1929, p. 221; SEWELL, 1929, p. 68; WILSON, 1932, p. 38; FARRAN, 1936. p. 80; MORI, 1937, p. 29, pl. 11; VERVOORT, 1947, p. 130.

Female. Forma *minor*: length, 0.74–0.82 mm. The head evenly rounded in lateral view; the line of demarcation between the head and the 1st thoracic segment is entirely undetectable. The abdomen is contained 3.7-times in the length of the caphalothorax; the proportional lengths of the abdominal segments and furca 26 : 16 : 13 : 26 : 19 = 100.

The 1st antenna exceeds the end of the abdomen by terminal one joint.

In the swimming legs the number of the outer marginal teeth on the 3rd joint of the exopodite of the 2nd to 4th legs are 8 9, 14 and 17 respectively; in the 2nd and 3rd legs the outer margin between the proximal and the distal spine of the 3rd joint of the exopodite bears two or three spine; these spines are entirely absent in the 4th leg.

The 5th leg 2-jointed; the 1st joint swollen; the 2nd joint 3.7-times as long as wide, and has the inner marginal seta which is little longer than the 2nd joint.

Remarks. The present specimen agrees well with the description and the figures of *P. parvus* given by SEWELL in the following: small in size; the proportional lengths of the abdomen to the cephalothorax; the armature of the exopodite of the 2nd to 4th legs. But the 1st antenna and the endspine on the 3rd joint of the exopodite of the 2nd–4th legs are longer in proportion than those given by SEWELL.

Female. Forma *major*: length, 0.92–0.98 mm. The head separates incompletely from the 1st thoracic segment, and is humped along the dorsal line about the middle. The abdomen is contained about 3.4–times in the length of the cephalothorax; the proportional lengths of the abdominal segments and furca are 27 : 16 : 14 : 25 : 18=100; the furcal rami about 2–times as long as wide.

The 1st antenna extends to the end of the abdomen; the terminal joint 1.3–times as long as the penultimate one.

In the swimming legs the proximal outer margin of the 3rd joint of the exopodite of 2nd to 4th legs have 13, 15 and 27 teeth respectively; the proportional lengths of the 3rd joint of the exopodite to the endspine are as follows:

	Re 3	forma *major* Spt.	teeth	Re 3	forma *minor* Spt.	teeth
2nd	100	114	13	100	130	8–9
3rd	100	95	15	100	106	14
4th	100	75	27	100	83	17

The 5th leg 2–jointed; the proximal joint much swollen; the 2nd joint 3.4–times as long as wide, ends in two unequal spines; the spine on the inner distal margin 1.4–times as long as the joint itself.

Remarks. The specimen differs from the foregoing in the following: larger in size; the dorsal line of the head humped; the armature of the exopodite of the 2nd to 4th legs; the proportional lengths of the endspine to the 3rd joint of the exopodite of the 2nd to 4th legs.

Male. Length, 0.84–0.96 mm. The head has a small transparent swelling on the mid-dorsal line about the opposite position to the base of the 1st antenna. The abdomen is contained about 2.0–2.7–times in the length of the cephalothorax; the proportional lengths of the abdominal segments and furca are 10 : 23 : 17 : 18 : 16 : 16 =100, measured laterally along the dorsal line.

The 2nd joint of the exopodite of the 2nd to 4th legs is dentate on the outer margin; the teeth on the 3rd joint of the exopodite of the 4th leg are finer than those of the female.

The right 5th leg 2–jointed; the left leg 5–jointed; the terminal joint is furnished with two short spines.

Remarks. Two size groups are also detectable in the male of *P. parvus*; the small-sized measured from 0.84–0.88 mm; the large-sized measured from 0.88–0.96 mm;

they differ only in size; the former has somewhat longer abdomen than that of the latter.

Occurrence. The common species in the surface layer in the Izu rigion.

Distribution. The species is widely distributed in the warm regions of the oceans.

Genus *Acrocalanus* GIESBRECHT, 1888

Four species belonging to this genus have been recorded from the Japanese waters, of which three species occured in the present collection: *A. gracilis* GIESBRECHT, *A. longicornis* GIESBRECHT and *A. gibber* GIESBRECHT. The genus *Acrocalanus* differs from *Paracalanus* in the following: short abdomen; long 1st antenna; the 4th thoracic segment separates incompletely from the 5th; the armature on the joints of the exopodite of the 2nd to 4th legs; the distal outer margin of the endopodites end in a small spine; the female 5th leg absent or rudimentary; the male 5th legs present only on the left side. The male specimens of *Acrocalanus* resemble so closely each other that it is troublesome to connect the males with the corresponding females. In the present collection, the male specimens are represented by three species *A. gracilis*, *A. longicornis* and *A. gibber*.

Acrocalanus gracilis GIESBRECHT

Acrocalanus gracilis GIESBRECHT, 1892, p. 171, t. 6, 10; WOLFENDEN, 1906, p. 1000; A. SCOTT, 1909, p. 29; SEWELL, 1912, p. 395, 359; SEWELL, 1929, p. 79; FARRAN, 1936, p. 81; MORI, 1937, p. 31, pl. 12; VERVOORT, 1947, p. 134.

Female. Length, 1.25–1.32 mm. The head is evenly rounded; the line of demarcation between the head and the 1st thoracic segment very slight. The abdomen is contained 3.8-times in the length of the cephalothorax; the abdominal segments and furca in the proportional lengths 31 : 10 : 10 : 24 : 24 = 100; the furcal rami about 1.7-times as long as wide.

The 1st antenna extends beyond the end of the abdomen by distal three or four joints.

In the swimming legs the basal joints, the exopodite and endopodite are furnished with spinule, but the distal joint of the exopodite of the the 3rd leg has spinules only on the proximal half of the outer margin. The proportional lengths of the 3rd joint of the exopodite to the endspine in the 2nd to 4th legs are 94 : 100, 69 : 100 and 62 : 100 (length of the 3rd point taken as 100). The armature of the outer margin of the 2nd and 3rd joint of the 2nd to 4th legs as follows:

		2nd leg	3rd leg	4th leg
2nd joint		9	9	15
3rd joint	proximal part	8	10	16
	distal part	6	0	14

The 5th pair of legs are, in most cases, absent; some of the specimen had a rudimentary 3-jointed 5th leg on the left side.

Occurrence. A vertical haul from the depth 300 m to the surface contained 19 females.

Distribution. The species appears to be distributed in the tropical and subtropical regions of the oceans.

Acrocalanus longicornis GIESBRECHT

Acrocalanus longicornis GIESBRECHT, 1892, p. 171, t. 6, 10; WOLFENDEN, 1906, p. 1000; A. SCOTT, 1909, p. 28; SEWELL, 1912, p. 358; FARRAN, 1929, p. 222; SEWELL, 1929, p. 82; FARRAN, 1936, p. 80; MORI, 1937, p. 31, pl. 12; VERVOORT, 1947, p. 33.

Female. Length, 1.14–1.28 mm. The head incompletely separates from the 1st thoracic segment; the dorsal profile of the head slightly humped. The abdomen is contained 3.8-times in the length of the cephalothorax; the proportional lengths of the abdominal segments and furca are 33 : 13 : 10 : 20 : 23 = 100; the furcal rami about 2-times as long as wide; the genital segment swollen below.

The 1st antenna extends beyond the end of the abdomen by distal five joints.

In the swimming legs the armature on the outer margin of the 2nd and 3rd joints of the exopodite, and the proportional lengths of the 3rd joint to the endspine of the 2nd to 4th legs are as follows:

		2nd leg	3rd leg	4th leg
Exopod 2		2	7–8	13
Exopod 3	proximal part	5	8–9	14–15
	distal part	5	0	43
Endspine		100	100	100
Exopod 3		86	61	56

The 5th leg absent; but some of the specimen has a rudimentary 3-jointed leg on the left side.

Occurrence. A vertical haul from the depth 300 m to the surface contained 10 female mixed with the foregoing.

Distribution. The species appears to have the similar distribution as that of *A. gracilis*, and has been recorded from the tropical and sub-tropical regions of the oceans.

Acrocalanus gibber GIESBRECHT

Acrocalanus gibber GIESBRECHT, 1892, p. 171, t. 6, 10; WOLFENDEN, 1906, p. 1003; A. SCOTT, 1909, p. 29; SEWELL, 1912, p. 315; SEWELL, 1914, p. 210; GURNEY, 1927, p. 147, fig. 18; SEWELL, 1929, p. 80; FARRAN, 1936, p. 81; MORI, 1937, p. 32, pl. 12; VERVOORT, 1947, p. 136.

Female. Length, 1.13 mm. The dorsal outline of the head is much humped; the separation of the head from the 1st thoracic segment is clearly made out. The abdomen is contained 3.85-times in the length of the cephalothorax; the abdomen 4-segmented, the segments and furca in the proportional lengths 31 : 11 : 10 : 25 : 23=100; the furcal rami about 2.3-times as long as wide.

The 1st antenna exceeds the end of the abdomen by terminal two joints.

The armature on the 2nd and 3rd joints of the exopodite of the 2nd to 4th legs, and the proportional lengths of the exopod 3 to the endspine are as follows:

	2nd leg	3rd leg	4th leg
Exopod 2	7	9	14
Exopod 3 {proximal part	9	10	16
Exopod 3 {distal part	4	0	5
Endspine	100	100	100
Exopod 3	78	64	55

In the 4th leg the proximal part of the outer margin of the 3rd joint of the exopodite is much longer than the distal part of the same joint.

Occurrence. The species is rare in the Izu region; only one specimen was collected in winter of 1934.

The male specimens of the genus *Acrocalanus* are closely related each other, and differ only in minute points of structure. In the present collection three representatives of the adult male were obtained. In the following I give a table, showing some structural differences in the males and females described by SEWELL:

Species	*A. gracilis*			*A. longicornis*			*A. gibber*		
Total length (mm) ♂ male	0.80–1.0			0.95–1.0			0.943		
Proportional lengths									
Abdominal segm. and furca	13 : 26 : 17 : 15 : 16 : 13			12 : 22 : 15 : 15 : 20 : 16			13 : 25 : 17 : 17 : 16 : 12=100		
Joints of 5th leg	25 : 13 : 24 : 20 : 18			23 : 14 : 25 : 20 : 18			26 : 12 : 25 : 22 : 15=100		
	2nd	3rd	4th leg	2nd	3rd	4th leg	2nd	3rd	4th leg
Exopod 2	22	25	25	22	27	27	24	26	27
Exopod 3 {dist.	19	20	17	22	21	20	19	20	18
Exopod 3 {prox.	20	22	28	20	21	24	20	21	23
Endspine	39	33	30	36	31	29	39	33	32
No. of spine									
Exopod 2	10–11	11–12	17	8–10	12	16–20	11	13	20–22
Exopod 3 {dist.	8	14	17	7–9	15	15–20	7–8	14–15	10–11
Exopod 3 {prox.	11	15	22	9–10	13	23–25	10	15	25

Total length (mm) female	1.0			1.0			0.81		
Proportional lengths									
Exopod 2	28	26	26	21	27	27	23	27	28
Exopod 3 dist.	19	20	22	22	24	24	20	22	29
Exopod 3 prox.	20	22	23	20	20	22	20	21	27
Endspine	39	32	29	37	29	27	31	30	26

The male specimens of the present collection have the following figures:

Total length (mm)	1.20–1.24			0.955			1.40		
Proportional lengths									
Abdomen segm. and furca	12 : 25 : 18 : 16 : 18 : 11			11 : 24 : 17 : 15 : 20 : 13			13 : 25 : 17 : 17 : 15 : 13=100		
5th leg	24 : 22 : 21 : 17 : 16			23 : 25 : 20 : 17 : 15			18 : 26 : 22 : 18 : 16=100		
Exopod 2	23	27	27	22	27	27	24	25	27
Exopod 3 dist.	18	18	19	18	20	20	17	19	19
Exopod 3 prox.	21	24	25	23	23	20	22	23	22
Endspine	38	31	29	31	30	26	38	33	32

Comparing these figures each other, I am inclined to regard the large specimen (1.20–1.24 mm) as the male of *A. gracilis*, and the small specimen (0.955 mm) as the male of *A. longicornis*, and the largest specimen (1.40 mm) as the male of *A. gibber*.

Acrocalanus gracilis

Male. Length, 1.20–1.24. The proportional lengths of the cephalothorax to the abdomen are 99 to 36, so the abdomen is contained 2.75-times in the length of the cephalothorax. The abdominal segments and furca, measured in dorsal aspect, have the proportional lengths 12 : 25 : 18 : 16 : 18 : 11 = 100; the furcal rami 1.3-times as long as wide.

The 1st antenna exceeds beyond the posterior margin of the last thoracic segment by terminal three joints; the proportional lengths of the joints measured along the posterior margin are:

Joint	1–6	7–8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	286	58	22	27	31	31	40	40	45	45	45	45	45	45	45	45	45	40	27=1000.

The proportional lengths and the armature of the 2nd to 4th legs are as tabulated above.

The immature specimen in the copepodid stage V were also taken associated with the adult males and females. The total length of the specimen measured 1.07–

1.16 mm. The proportional lengths of the 2nd and 3rd joints of the exopodite to the endspine, and the number of spines on the outer margin of the 2nd and 3rd exopod of the 2nd to 4th legs are as follows:

Proportional lengths of the joints		2nd leg	3rd leg	4th leg
Exopod 2		21	24	24
Exopod 3	prox.	21	24	28
	dist.	18	18	18
Endspine		40	34	30
Number of spines on the outer margin				
Exopod 2		1	5	6
Exopod 3		5	6	9

The left 5th leg 4-jointed.

Acrocalanus longicornis

Male. Length, 0.955 mm. The proportional lengths of the cephalothorax to abdomen are 71 : 26, so the abdomen is contained 2.85-times in the length of the cephalothorax. The forehead narrowly rounded; a small transparent swelling is observed on the mid-dorsal line of the cephalon. The line of demarcation between the 4th and 5th thoracic segment can be clearly made out in lateral aspect. The rostrum is rather stout and short. The proportional lengths of the abdominal segments and furca, measured along the dorsal, are 11 : 24 : 17 : 15 : 20 : 13=100; the furcal rami 1.4-times as long as wide.

The 1st antennae reach back beyond the posterior margin of the last thoracic segment by the extent of the terminal three joints; the proportional lengths of the joints measured along the posterior margin are:

Joint	1-6	7-8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	23	25
	274	63	29	34	35	40	40	46	46	46	47	46	46	46	46	46	46	40	29=1000.

The proportional lengths of the joints of 2nd to 4th legs are as tabulated above. The left 5th leg has the joints in the proportional lengths 23 : 25 : 20 : 17 : 15=100; the endspine of the 5th joint about 2/5 the length of the distal joint.

The immature male specimens in the copepodid stage V, measured 0.96-0.97 mm. The specimen has the swimming legs similar in structure to those of the female; the proportional lengths of the various joints and the number of the outer marginal spine on the 2nd and 3rd joint of the exopodite of the 2nd to 4th legs are as follows:

Proportional lengths of the joints		2nd leg	3rd leg	4th leg
Exopod 2		22	26	27
Exopod 3	prox.	20	22	26
	dist.	19	20	20
Endspine		37	32	27
Number of outer marginal spines				
Exopod 2		1	7	8
Exopod 3	prox.	5	6	10
	dist.	5	0	5

The endspine of the last joint of the left 5th leg is longer than that of the immature male of *A. gracilis*.

Occurrence. The adult males of *A. gracilis* and *A. longicornis* were collected in the vertical hauls in Sept. 1937 associated with the adult females.

Acrocalanus gibber

Male. Length, 1.40 mm. The head is evenly rounded; there is a small transparent swelling on the mid-dorsal line about the anterior one third of the head; the line of fusion between the head and the 1st thoracic segment can be made out in lateral aspect. The abdomen is contained 2.8-time in the length of the cephalothorax. The abdominal segments and furca in the proportional lengths 13 : 25 : 17 : 17 : 15 : 13 = 100.

The 1st antennae reach back beyond the posterior margin of the cephalothorax by the extent of two terminal joints.

The proportional lengths of the various joints, and the armature on the 2nd and 3rd exopod of the 2nd to 4th legs are as follows:

Proportional lengths of the joints		2nd leg	3rd leg	4th leg
Exopod 2		24	25	27
Exopod 3	prox.	22	23	22
	dist.	17	19	19
Endspine		38	33	32
Number of teeth				
Exopod 2		15	17	34
Exopod 3	prox.	13	15	29
	dist.	9	15	25

The 3rd joint of the exopodite of the 1st leg has a longitudinal row of spinules on the proximal part along the outer margin.

The 5th leg has the following proportional lengths, commencing from the base: 18 : 26 : 22 : 18 : 16 = 100.

The present specimen agrees fairly well with SEWELL's description of the male of *A. gibber*, but differs from it in the following: larger in size; the length of the 1st antenna; the proportional lengths of the 5th joints of the left 5th leg.

Occurrence. A single specimen in winter of 1934 from the surface.

Genus *Calocalanus* GIESBRECHT, 1888

From the Japanese waters only one species *Calocalanus pavo* (DANA) has been reported. In the present collection the following five species were detected: *C. pavo* (DANA), *C. plumulosus* (CLAUS), *C. styliremis* GIESBRECHT, *C. pavoninus* FARRAN, *C. gracilis* sp. nov.; the last species appears to be undescribed, and to which I give the name *C. gracilis*.

The general character of the genus resembles that of *Paracalanus*, but it is easily distinguished from the former in the segmentation, and the shape of the abdomen, and the proportional lengths of the joints of the 1st antenna.

Calocalanus pavo (DANA)

Calocalanus pavo GIESBRECHT, 1892, p. 175, t. 1, 4, 9; WOLFENDEN, 1906, p. 999; A. SCOTT, p. 30; SEWELL, 1914, p. 214; FARRAN, 1929, p. 222; SEWELL, 1929, p. 89; WILSON, p. 1932, p. 39; FARRAN, 1936, p. 83; MORI, 1937, p. 33, pl. 13. VERVOORT, 1947, p. 138.

Length. Female, 1.23 mm.

Occurrence. The species is not common here; one adult female was obtained in a vertical haul from 50 m to the surface in August 1937.

Distribution. The species is widely distributed in the tropical and subtropical regions of the Pacific, Atlantic and Indian Ocean.

Calocalanus plumulosus (CLAUS)

(Fig. 7, *d–e*)

Calocalanus pulmulosus GIESBRECHT, 1892, p. 176, t. 3, 9, 36; WOLFENDEN, 1906, p. 999; A. SCOTT, 1909, p. 31; SEWELL, 1914, p. 214; FARRAN, 1929, p. 223; SEWELL, 1929, p. 89; WILSON, 1932, p. 41; FARRAN, 1936, p. 83.

Female. Length, 1.00–1.18 mm. The body slender; the abdomen is contained about 5.2-times in the length of the cephalothorax; the abdomen 3-jointed; the furcal rami symmetrical.

The 1st antenna 24-jointed, exceeds the end of the furca; the terminal joint is 2-times as long as the penultimate one. The 5th leg somewhat differs from that figured by GIESBRECHT: the inner marginal seta on the apex of the 3rd joint is very short; the 3rd joint is about 8-times as long as wide, whereas it is 9-times in the GIESBRECHT's figure. Some specimens had slightly asymmetrical 5th pair of legs.

Male. Length, 0.88–0.90 mm. The head separate from the 1st thoracic segment and so is the 4th from the 5th. The abdomen is contained about 3.4-times in the length of the cephalothorax; the proportional lengths of the abdominal segments and furca are 17 : 21 : 19 : 17 : 15 : 12 = 100; the furcal rami about as long as wide.

The 1st antenna 21-jointed, exceeds the end of the cephalothorax by distal five joints.

The 1st leg with an inner marginal seta on the 1st basal joint. The 3rd leg has three groups of spinules on the posterior surface of the 3rd joint of the endopodite; the 2nd joint of the exopodite has a row of leaf-like spines on the outer margin, and also on the inner margin about proximal one-third of the joint. The 4th leg has a group of spinules on the posterior surface of the 3rd joint of the endopodite.

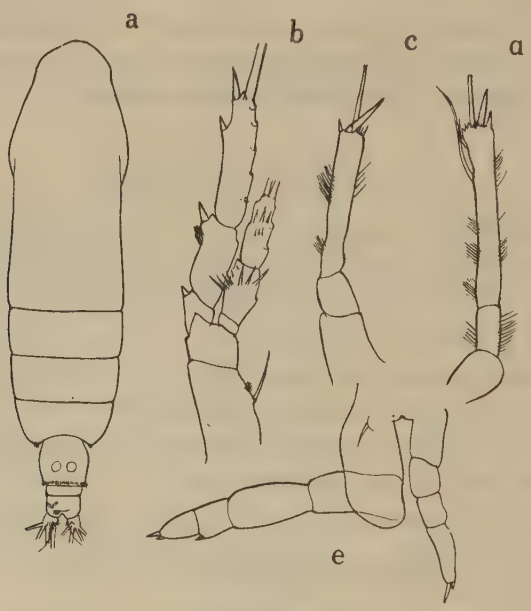

Fig. 7. *Calocalanus plumulosus* (CLAUS).
a, Female: dorsal aspect; *b*, 3rd leg; *c*, 5th leg; *d*, 5th leg, the other specimen; *e*, male: 5th pair of legs.

The 5th legs are similar in structure to those of *Calocalanus pavo*; the left leg 5-jointed; the right leg 4-jointed; the proportional lengths of the joints of the legs as follows:

	1	2	3	4	5
left leg	39	21	23	9	8 = 100
right leg	39	13	17	31	= 100

The proportional lengths of the corresponding joints in C. *pavo*, measured from the GIESBRECHT's figure are:

left leg	30	15	22	22	12 = 100
right leg	36	18	18	28	= 100

Occurrence. Common in the surface layer in summer season.

Distribution. The species is widely distributed in the tropical and sub-tropical regions of the oceans.

Calocalanus styliremis GIESBRECHT

(Fig. 8, *a–b*)

Calocalanus styliremis GIESBRECHT, 1892, p. 176, t. 3, 9, 36; WOLFENDEN, 1906, p. 999; G. O. SARS, 1925, p. 27; FARRAN, 1926, p. 234; FARRAN, 1929, p. 222; SEWELL, 1929, p. 89; FARRAN, 1936, p. 83.

There are two size groups in the female of *Calocalanus styliremis*: the small specimen can easily be referred *C. styliremis* and the large resembles *C. contractus* FARRAN. The small specimen measured 0.60–0.69 mm, and the large 0.75–0.84 mm. The small specimen is robust; the cephalothorax is 2.6-times as long as wide. The large specimen is rather slender and the cephalothorax is about 3-times as long as wide. The abdomen is contained about 5.3-times in the length of the cephalothorax in both specimens. The furca as long as wide. The 1st antenna exceeds the end of the furca by distal five joints; the joints 1 and 2 are separate; the last joint is, in small specimen, less than 2-times the length of the penultimate (21 : 12), whereas it is 2-times in the large specimen (30 : 14–16). The proportional lengths of the outer margin of the 3rd joint of the exopodite and the endspine of the 3rd leg are nearly the same in both specimens:

	proximal	distal	endspine
small-sized specimen	31	11	58
large-sized specimen	31	10	59

The 5th legs of the small specimen resemble those figured by GIESBRECHT; the distal joint is about 4-times as long as wide, and has a slight swelling on the proximal part. In the large specimen the distal joint is about 3-times as long as wide, and carries four spines at the base of the terminal spine.

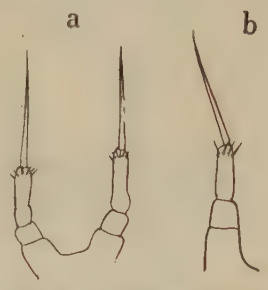

Fig. 8. *Calocalanus styliremis* GIESBRECHT.
a, Female: 5th pair of legs; *b*, 5th leg of the large specimen.

According to GIESBRECHT the group of the spine on the posterior surface of the endopodite of the 3rd and 4th legs are constant in number, but they are three in the small specimen, and two in the large specimen.

Occurrence. Common, but only the female specimen has been taken in the Izu region.

Distribution. The species has a wide distribution in the tropical and sub-tropical regions of the oceans.

Calocalanus pavoninus FARRAN

(Fig. 9, *a–c*)

Calocalanus pavoninus FARRAN, 1936, p. 83, fig. 3.

Female. Length, 0.72 mm; cephalothorax, 0.62 mm, abdomen, 0.10 mm; so the abdomen is contained 6.2-times in the length of the cephalothorax; the cephalothorax 2.9-times as long as wide. The proportional lengths of the abdominal segments and furca are 43 : 33 : 34 = 100; the genital segment 2-times as wide as long, and has a marked ventral swelling; the anal segment 2-times as wide as long; the furcal rami about as long as wide.

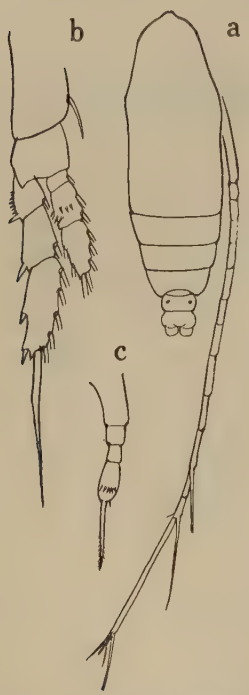

Fig. 9. *Calocalanus pavoninus* FARRAN. *a*, Female: dorsal aspect; *b*, 2nd leg; *c*, 5th leg.

The 1st antennae exceed the end of the abdomen by distal seven joints; the last joint is about 6-times as long as the penultimate one. The 1st leg has 3-jointed exopodite and endopodite. In the 2nd leg the 1st joint of the exopodite has about six teeth-like spines on the outer margin; the 2nd joint of the endopodite has rows of spinules on the posterior surface; the inner marginal seta absent on the 1st basal joint. In the 3rd and 4th legs the 2nd and 3rd joints of the endopodite have each groups of spinules on the posterior surface. The 5th leg 4-jointed; the terminal joint is gradually inflated at the distal end, and about as long as the length of the two basal joints taken together; a line of fusion can be clearly seen at the proximal one-third of the joint; there are a minute spinule at the base of the terminal seta and a transverse row of spinules.

Remarks. The present specimen agrees closely with *C. pavoninus* FARRAN, except minute differences in the structure of the 3rd and 5th legs.

Occurrence. Three adult females in the surface collection in August 1937.

Distribution. The species has been recorded from the Great Barrier Reef region.

Calocalanus gracilis TANAKA

(Fig. 10, *a–e*)

Calocalanus gracilis TANAKA, 1956, p. 7, fig. 3, *a–h*.

Female. Length, 0.62 mm. The body slender; the cephalothorax is 3.5-times as long as wide; the abdomen is contained 4.8-times in the length of the cephalothorax. The abdomen 3-jointed; the proportional lengths of the segments and furca are 40 : 8 : 32 : 20 – 100.

The distal joints of the 1st antenna missing; the proportional lengths of the joints 1–15 in 0.01 mm are as follows:

Joint	1	2	3	4	5	6	7	8–9	10	11	12	13	14	15
	3.2	4.1	2.3	1.8	2.3	2.3	2.3	3.2	1.8	1.8	2.0	2.3	2.7	2.7

The swimming legs small. The 1st leg has 2-jointed exopodite and 1-jointed endopodite; the 1st basal joint has small spines on the inner distal margin; the proximal joint of the exopodite has a seta on the inner margin about the middle. The 2nd to 4th legs have each 3-jointed exopodite and endopodite; the 1st joint of the exopodite of 2nd leg has four small spines on the outer margin at the base of the outer edge spine; in the 3rd and 4th legs the 2nd and 3rd joints of the endopodite are devoid of groups of spinule on the posterior surface; the leaf-like spines of the 2nd and 3rd joints of the exopodite also absent. The 5th leg 3-jointed; the terminal joint about as long as the 1st, and bears an endspine which is of equal length to the three joints taken together, and have a very minute spine on the outer distal corner of the joint.

Remarks. The specimen resembles *C. tenuis* FARRAN, when viewed from the dorsal, but its small size and the structure of the swimming legs separate the present specimen from the former.

Occurrence. One adult female in the vertical haul from the depth 50 m to the surface in August, 1937. A. FLEMINGER recorded in 1955 the occurrence of the species from the Gulf of Mexico. The description and figures were given in "Breviora," issued from the Museum of Comparative Zoology of Harvard University.

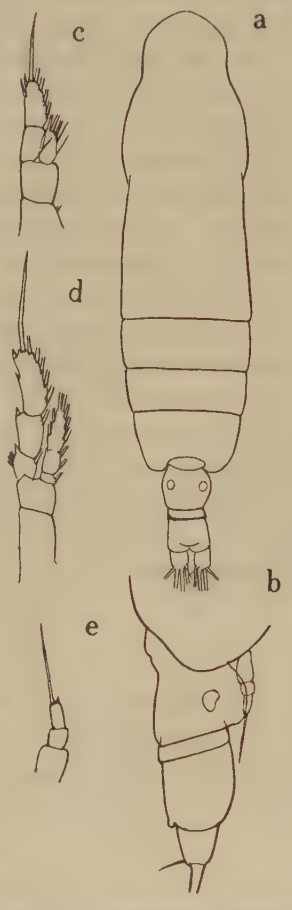

Fig. 10. *Calocalanus gracilis* TANAKA. *a*, Female: dorsal aspect; *b*, 1st thoracic segment and abdomen, lateral aspect; *c*, 1st leg; *d*, 2nd leg; *e*, 5th leg.

Family PSEUDOCALANIDAE

The family, at present, comprises eight genera, namely *Clausocalanus* GIESBRECHT, *Ctenocalanus* GIESBRECHT, *Pseudocalanus* BOECK, *Spinocalanus* GIESBRECHT, *Microcalanus* SARS, *Mimocalanus* FARRAN, *Monacilla* SARS, *Drepanopsis* WOLFENDEN and *Tanyrhinus* FARREN. These genera have the 1st antenna composed of 24 joints; the joints 8 and 9 are completely fused, except those of *Mimocalanus*, *Monacilla* and *Tanyrhinus*. In the swimming legs the exopodite of the 1st to 4th legs are 3-jointed;

the endopodite of the 1st leg 1-jointed, of the 2nd leg 2-jointed, of the 3rd and 4th legs 3-jointed. The number of the outer-edge spine on the terminal joint of the exopodite of the 2nd to 4th legs is three, and the number of the inner marginal setae of the same joints is five. The number of the setae on the distal joint of the endopodite of the 2nd to 4th legs is five, except *Spinocalanus*, *Monacilla* and *Tanyrhinus*; in these three genera the 3rd and 4th legs have four inner marginal setae, whereas in the remaining genera, there are six setae on the terminal joint of the endopodite. In the 1st leg the 1st to 3rd joints of the exopodite have each an outer-edge spine respectively, except *Microcalanus* and *Mimocalanus* which have no seta on the 1st joint of the exopodite, and *Tanyrhinus* which has no outer-edge spine on the 1st and 2nd joints of the exopodite. The armature on the face of the swimming legs are observed in *Spinocalanus*, *Monacilla* and *Drepanopsis*. The female 5th legs are absent or rudimentary, except in *Drepanopsis*. The male 5th legs are, in most cases, uniramose, except in *Spinocalanus* and *Monacilla* which have biramose 5th legs.

Considering the relationship among these genera, it is possible to divide them into the following four groups: (1) *Clausocalanus*, *Ctenocalanus*, *Pseudocalanus* and *Drepanopsis* and *Microcalanus*; (2) *Mimocalanus*; (3) *Spinocalanus* and *Monacilla*; (4) *Tanyrhinus*. FARRAN suggests that *Mimocalanus* is closely allied to *Paracalanus* in the segmentation of the 1st antenna.

Beside these genera, I found from the deep water of Suruga Bay, a specimen which had the swimming legs similar in structure to those of *Spinocalanus*. It differs from the genera given above also in the structure of the male 1st antenna, to which I propose a new generic name *Ryocalanus*.

Genus *Clausocalanus* GIESBRECHT, 1888

At the present time three species have been recorded from the Japanese waters; all of them appeared in the collection, namely; *Clausocalanus arcuicornis* (DANA), *C. furcatus* (BRADY) and *C. pergens* FARRAN.

Clausocalanus arcuicornis (DANA)

(Fig. 11, *a–d*)

Clausocalanus arcuicornis GIESBRECHT, 1892, p. 186, t. 2, 10, 36; ESTERLY, 1905, p. 142; WOLFENDEN, 1906, p. 999; A. SCOTT, 1909, p. 32; SEWELL, 1912, p. 360; G. O. SARS, 1925, p. 27; FARRAN, 1926, p. 237; GURNEY, 1927, p. 150; FARRAN, 1929, p. 223; SEWELL, 1929, p. 90; WILSON, 1932, p. 42; FARRAN, 1936, p. 82; TANAKA, 1937, p. 252; MORI, 1937, p. 34, pl. 13, 14; VERVOORT, 1947, p. 140.

Female. There are two size groups in the female of *C. arcuicornis*. SEWELL described these two forms from the Indian waters. FARRAN reported these two forms in "Copepoda of the Great Barrier Reef Expedition" too. I have detected the two forms from Suruga Bay.

Forma *minor*. Length, 1.18–1.29 mm. The abdomen is contained 3.0–3.4-times in the length of the cephalothorax; the rostrum is slender, and bents slightly backwards. The 5th legs (Fig. 11 *a*) are quite different from those of forma *major* (Fig. 11 *b*).

Forma *major*. Length, 1.41–1.52 mm; the abdomen is contained 2.7–2.8-times in the length of the cephalothorax; the rostrum is stout and rather straight.

Male. Two different types are observed in the male specimen; the difference lies in the structure of the 5th legs; the one, measuring 1.23 mm, has the 5th legs as those hitherto been described (Fig. 11 *b*); the other measuring 1.07–1.29 mm in total length, have a very slender distal joint in the right 5th leg (Fig. 11 *d*). The latter specimens occurred both from the surface and deep waters; the surface specimens are slightly larger (1.23–1.29 mm) than those of the deep waters (1.07 mm).

Occurrence. Very common.

Distribution. The species has a wide distribution in the warm and temperate regions of the oceans.

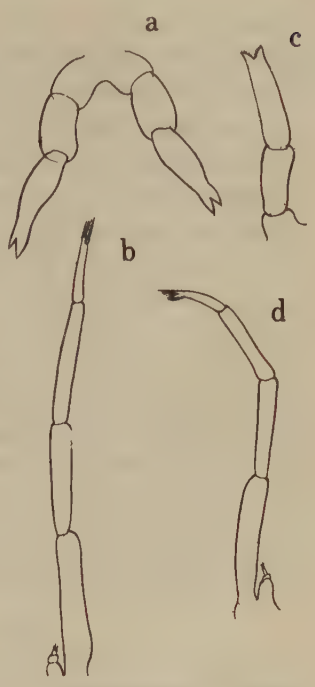

Fig. 11. *Clausocalanus arcuicornis* Dana. *a*, Female, forma *major*: 5th pair of legs; *b*, Male: 5th pair of legs; *c*, Female, forma *minor*: 5th legs; *d*, Male: 5th pair of legs.

Clausocalanus furcatus (Brady)

Clausocalanus furcatus Giesbrecht, 1892, p. 186, t. 36; Wolfenden, 1906, p. 1007; A. Scott, 1909, p. 32; Sewell, 1914, p. 215; Sars, 1925, p. 28; Farran, 1929, p. 225; Sewell, 1929, p. 93; Farran, 1936, p. 81; Mori, 1937, p. 35, pl. 14; Vervoort, 1947, p. 144.

Length. Female, 1.08–1.18 mm. Male, 0.85 mm.

Occurrence. One of the most common species in the Izu region.

Distribution. The species has a wide distribution in the warm region of the oceans.

Clausocalanus pergens Farran

Clausocalanus pergens Farran, 1926, p. 239; Mori, 1937, p. 35, pl. 14.

Female. Length, 0.99 mm. The abdomen is contained 2.8-times in the length of the cephalothorax; the proportional lengths of the abdominal segments and furca are 34 : 21 : 19 : 12 : 14 = 100. The rostrum slender, slightly bent backwards. The 1st

antenna extends to the middle of the genital segment. In the 2nd leg the teeth on the distal margin of the 2nd basal joint are long and slender. The 5th leg ends in two closely-set points.

Occurrence. One adult female from the vertical haul from 1000 m to the surface in Suruga Bay on 25 June, 1937.

Distribution. The species appears to have a wide distribution, and has been recorded from the Bay of Biscay, but not yet from the Indian Seas. In Japan it has been recorded from the warm currents.

Genus *Ctenocalanus* GIESBRECHT, 1888

The genus comprises only one species, *Ctenocalanus vanus* GIESBRECHT. The genus is closely allied to *Clausocalanus*, but is easily distinguished from the former by the ctenate form of the outer edge spine on the 3rd joint of the exopodite of 3rd and 4th leg. In the male the 1st leg has an outer-edge spine only on the terminal joint of the exopodite. *Ctenocalanus longicornis* described by MORI appears to be a synonym of *C. vanus* GIESBRECHT.

Ctenocalanus vanus GIESBRECHT

Ctenocalanus vanus GIESBRECHT, 1892, p. 194, t. 10, 36; ESTERLY, 1924, p. 90; FARRAN, 1929, p. 226; FARRAN, 1936, p. 85; TANAKA, 1937, p. 253; *C. longicornis* MORI, 1937, p. 37, pl. 15.

Length. Female, 1.18–1.27 mm. Male, 1.25–1.40 mm.

Remarks. MORI reported a specimen which has no 5th pair of legs from Japanese waters. The immature male has 4-jointed abdomen; the ctenate outer marginal spines are found in the 3rd and 4th legs; they measured 1.09–1.14 mm.

Occurrence. The species has been collected both from the surface and deep waters, but it is rather abundant in the deep layers.

Distribution. The species is widely distributed in the warm and temperate regions of the Pacific, Atlantic, Red Sea, Mediterranean Sea, San Francisco Bay, La Jolla and also from the Antarctic region, but not from the Indian and Malayan regions.

Genus *Pseudocalanus* BOECK, 1872

Two species have been recorded from the Japanese waters: *Pseudocalanus minutus* (KRÖYER) and *P. gracilis* SARS. One of them appeared in the collection; the species is the inhabitant of northern cold waters.

Pseudocalanus minutus (KRÖYER)

Pseudocalanus elongatus GIESBRECHT, 1892, p. 197, t. 10; SARS, 1908, p 20; SATO, 1913, p. 18; *P. minutus* WITH, 1915, p. 57; WILSON, 1932, p. 43; MORI, 1937, p. 36, pl. 15.

Length. Male, 1.30 mm.

Occurrence. A single male specimen was obtained in a vertical haul from the depth 1000 m to the surface in Sagami Bay, Nov., 1937.

Distribution. The species has a wide distribution in the temperate waters of the North Pacific and Atlantic. It has not been recorded from the Indian Seas.

Genus *Microcalanus* G.O. Sars, 1901

The genus was created by Sars to accomodate a small Calanoid, taken in the Norwegian coast, named *Microcalanus pusillus*; then he added the second species, *M. pygmaeus*, which had been described as *Pseudocalanus pygmaeus*, taken in the Nansen's North Polar Expedition. These two species resemble so closely each other that the distinction between them is very unclear. The generic characters are as follows: the head fused with the 1st thoracic segment, so the 4th with the 5th; the rostrum small; the abdomen 4-jointed; the furca small; the 1st antenna 24-jointed; the oral parts as in *Pseudocalanus* except the slender maxillipede; the swimming legs as in *Pseudocalanus*; the 5th legs absent in the female; the male 5th legs asymmetrical, 6-jointed on the left side.

One species belonging to the genus was found from the intermediate layer of Sagami Bay.

Microcalanus pygmaeus G.O. Sars

(Fig. 12, *a–f*)

Microcalanus pygmaeus Giesbrecht, 1902, p. 000; *M. pusillus* Wolfenden, 1911, p. 000; *M. pygmaeus* With, 1915, p. 66; Farran, 1929, p. 226; Jespersen, 1934, p. 50.

Female. Length, 0.85 mm, cephalothorax 0.64 mm, abdomen 0.21 mm; the abdomen is contained 2.9-times in the length of the cephalothorax. The cephalothorax ovate; the greatest width about half the length of the cephalothorax. The head fused with the 1st thoracic segment; the last two thoracic segments are fused; the lateral corners of the last thoracic segment narrowly rounded at the apex. The rostrum pointed, directs slightly posteriorly. The abdomen 4 jointed; the segments and furca in the proportions 37 : 21 : 17 : 17 : 8 = 100; the genital segment rounded, about as long as wide, and produced considerably below; the furcal rami slightly longer than wide.

The 1st antenna 24-jointed, extends at least to the distal margin of the 3rd abdominal segment. The 2nd antenna has the exopodite longer than the endopodite (15 : 11); the 2nd joint of the exopodite has three setae; the 3rd has one seta. The mandible has the exopodite slightly shorter than the endopodite (4 : 5). The Maxilla and maxillipede are as those of *M. pusillus* illustrated by G. O. Sars.

In the 1st leg the outer marginal spines on the 2nd and 3rd joints long and curved inwards. In the 2nd leg the terminal spine of the exopodite about as long as

the combined lengths of the three joints of the exopodite taken together, and has 27 serrations; in the GIESBRECHT's specimen the number of the serration is about 40. The terminal spine of the exopodite of the 3rd leg broken off. The 4th leg abnormal: the exopodite 2-jointed, the 2nd basal joint has spinules on the distal inner margin at the base of the endopodite.

Remarks. The specimen, though resembles closely *M. pusillus*, differs from it in the length of the terminal spine, and the number of serration on the exopodite of the 2nd leg. *M. pygmaeus*, taken from the Antarctic, has comparatively long 1st antenna extending to the anterior margin of the anal segment and has the terminal spines of

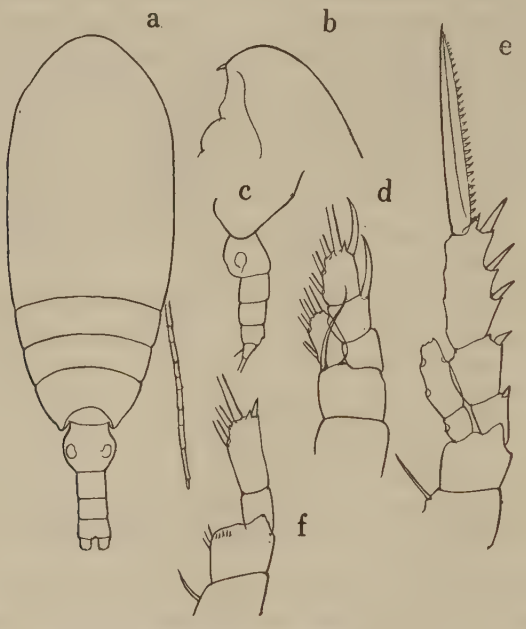

Fig. 12. *Microcalanus pygmaeus* G. O. SARS.
a, Female: dorsal aspect; *b*, head, lateral aspect; *c*, last thoracic segment and abdomen, lateral aspect; *d*, 1st leg; *e*, 2nd leg; *f*, abnormal 4th leg.

the exopodite of the 2nd to 4th legs longer and more finely toothed when compared with those of *M. pusillus* which has only 16 teeth on the terminal spine of the 2nd leg. *M. pygmaeus* was originally described by Sars from the specimen taken in the Arctic region. GIESBRECHT described the same species taken from the Antarctic regions. According to WOLFENDEN *M. pygmaeus* described by GIESBRECHT is not identical with *M. pygmaeus* G. O. SARS, but identical with *M. pusillus* G. O. SARS. SARS stated in his description that in *M. pusillus* the 1st antenna is much shorter than that of *M. pygmaeus*, scarcely extending beyond the end of the genital segment; the swimming legs of *M. pusillus* are less slender than those of *M. pygmaeus*; the terminal

joint of the exopodite of the 2nd to 4th legs comparatively broader, and has the apical spine remarkably dilated, cultrate in shape, and very coarsely serrated. The present specimen agrees more closely with the Antarctic specimen of *M. pygmaeus*, described by GIESBRECHT or FARRAN in the structure of the swimming legs than with *M. pusillus* G. O. SARS.

Occurrence. One female in the vertical haul from the depth 500 m to 300 m, in Sagami Bay.

Distribution. The species has a bipolar-epiplanktonic distribution. It has been recorded from the Arctic regions by G. O. SARS, WITH and JESPERSEN. From the Antarctic it has been reported by GIESBRECHT, WOLFENDEN and FARRAN. The species has not yet been recorded from the Indian and Malayan regions.

Genus *Mimocalanus* FARRAN, 1908

The genus was created by FARRAN to accomodate a number of species closely related to *Spinocalanus*. FARRAN described two species belonging to the genus, namely *Mimocalanus cultrifer* and *M. nudus*, one of which appeared in the collection. The genus is characterised by the followings: absence of rostral filament; the separation of the last two thoracic segments; the equal branches of the 2nd antenna; the large two-branched palp of the mandible, of which the inner being longer than the outer; the maxillae and maxillipede being of *Paracalanus* type; the 1st to 4th legs with jointing and setae as in *Spinocalanus* except that the outer-edge spine on the 1st joint of the exopodite of the 1st leg is absent; the terminal spine of the exopodite of the 2nd to 4th legs are of cultrate form; no spinules on the faces of the legs; the 5th leg absent in the female; the male 5th legs 5-jointed on left side, 4-jointed on the right.

Mimocalanus cultrifer FARRAN

(Fig. 13, *a–i*)

Mimocalanus cultrifer FARRAN, 1908, p. 23, pl. 1; FARRAN, 1926, p. 244; VERVOORT, 1947, p. 156.

Female. Length, 1.50 mm; cephalothorax, 1.11 mm, abdomen, 0.29 mm; the cephalothorax oblong ovate in dorsal view, contracts anteriorly and the frontal margin is vaulted; the head fused with the 1st thoracic segment; the last two thoracic segments separate, but the line of suture very faintly detectable; the lateral corners of the last thoracic segment narrowly rounded at the apex; the rostrum absent. The abdomen 4-jointed, contained 4 times in the length on the cephalothorax; the segments and furca in the proportions 49 : 16 : 13 : 19 : 13 = 100; the genital segment about as long wide and moderately produced ventrally; the furcal rami as long as wide.

The 1st antenna 24-jointed, exceeds the distal margin of the furca by last one or two joints; the proportional lengths of the joints measured along the posterior margin

as follows:

Joint	1	2	3	4	5	6	7	8–9	10	11	12	13	14	15	16	17
	47	35	18	18	23	23	23	52	35	35	41	47	52	52	52	52
	18	19	20	21	22	23	24	25								
	52	58	52	47	52	52	47	35 = 1000;								

Fig. 13. *Mimocalanus cultrifer* FARRAN.
a, Female: dorsal aspect; *b*, last thoracic segment and abdomen, lateral aspect; *c*, head, lateral aspect; *d*, 1st maxilla; *e*, 1st leg; *f*, 2nd leg; *g*, 1st basal joint of 4th leg; *h*, Male: last thoracic segment and abdomen, lateral aspect; *i*, 5th pair of legs.

the joints 8 and 9 are incompletely fused; the joints 10 and 11 are slightly inflated. The 2nd antenna with the exopodite about as long as the endopodite; the 1st and 2nd joints of the exopodite has 9 marginal setae. The mandible has the exopodite slightly shorter than the endopodite. The 1st maxilla slightly differs from that described by FARRAN; the outer lobe has 7 setae, of which the proximal one is short;

the exopodite has 11 setae; the endopodite has 7 : 4 : 3 setae; the 2nd basal joint has 5 setae; the 3rd inner lobe has 3, the 2nd lobe has 4, the 1st lobe has 10 setae; the setae on the inner lobes are strong. The 2nd maxilla has 6 setae in all on the joints of the endopodite, but they are unmodified. In the maxillipede the combined lengths of the joints of the endopodite much longer than the 1st basal joint; the 2nd basal joint short.

The 1st leg has 3-jointed exopodite and 1 jointed endopodite; the 1st joint of the exopodite has no outer-edge spine. The 2nd leg has 3-jointed exopodite and 2 jointed endopodite; the outer-edge spine on the 2nd joint of the exopodite is the longest; the terminal spine of the exopodite finely serrated; the 3rd joint of the exopodite has 5 inner marginal setae. The 3rd and 4th legs have each 3-jointed exopodite and endopodite. The 4th leg has a row of stiff hairs on the inner margin of the 1st basal joint at the base of the inner marginal seta.

Male. Length, 1.28 mm; cephalothorax 0.98 mm, abdomen 0.3 mm. The general appearance as those of the female. The abdomen 5-jointed, and is contained 3.25-times in the length of the cephalothorax; the segments and furca in the proportions 19 : 18 : 18 : 15 : 18 : 12 = 100.

The 1st antenna exceeds the end of the furca by last two joints. Mouth parts and the swimming legs as those of the female.

The 5th pair of legs asymmetrical: the right side 4-jointed, the left side 5-jointed; the distal joint has hairs on the inner margin.

Remarks. The specimens agree fairly well with *M. cultrifer* which FARRAN collected from the Irish Atlantic Slope.

Occurrence. Two females and a male from Sagami Bay in the vertical haul from the depth 500 to 300 m, Nov., 1937.

Distribution. The species has only been recorded from the deep water of the Irish Atlantic Slope, but its geographical distribution is extended to the Celebes Sea in the Snellius Expedition.

Genus *Spinocalanus* GIESBRECHT, 1888

There has only one species of the genus *Spinocalanus* been known in the Japanese waters, namely *Spinocalanus abyssalis* from the deep water of Suruga Bay. The present collection added four more species: *Spinocalanus spinosus* FARRAN, *S. magnus* WOLFENDEN *S. angusticeps* G. O. SARS and *S. longipes.* The last species has not yet been described, and I propose a new name *longipes.*

Spinocalanus abyssalis GIESBRECHT

Spinocalanus abyssalis GIESBRECHT, 1892, p. 209, t. 13, 36; SARS, 1903, p. 22, 157; WITH, 1915, p. 69; FARRAN, 1926, p. 242; FARRAN, 1929, p. 227; JESPERSEN, 1934, p. 51; TANAKA, 1937, p. 253, fig. 4; VERVOORT, 1947, p. 147.

Remarks. The Atlantic specimen of *S. abyssalis* varies in size from 0.95 to 1.86 mm. FARRAN proposed a new variety name, var. *pygmaeus*, for the small form measuring 0.95–1.1 mm. GIESBRECHT's specimens measured 1.1–1.25 mm ; WITH's 1.73–1.86 mm ; FARRAN's 0.95–1.86 mm ; my specimen 1.01–1.93 mm.

Occurrence. Both size groups occurred commonly in the waters below 200 m.

Distribution. According to FARRAN the small form is less than half as plentiful as the large form above 300 fathoms, but below that depth it becomes much more numerous. *S. abyssalis* has been said to be of Arctic origin, and widely distributed in the deep waters of the oceans but it has not been recorded from the Indian and Malayan regions.

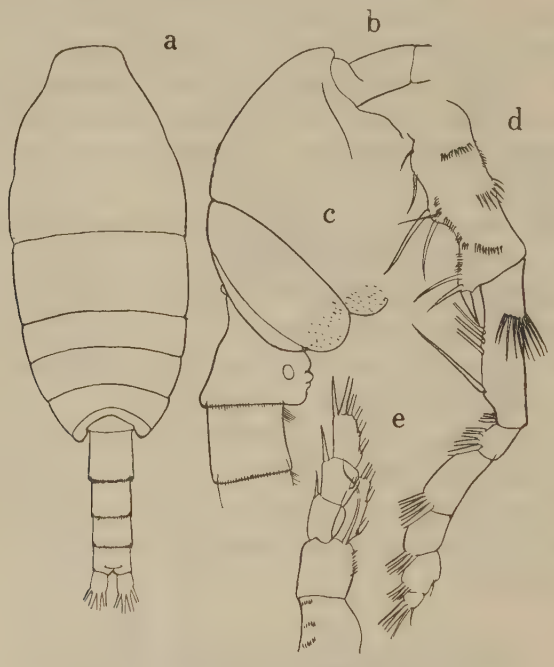

Fig. 14. *Spinocalanus spinosus* FARRAN.
a, Female : dorsal aspect ; *b*, head, lateral aspect ; *c*, last thoracic segment and genital segment, lateral aspect ; *d*, maxillipede ; *e*, 1st leg.

Spinocalanus spinosus FARRAN

(Fig. 14, *a–e*)

Spinocalanus spinosus FARRAN, 1908, p. 27 ; *Spinocalanus horridus* WOLFENDEN, 1911, p. 216 ; *S. spinosus* FARRAN, 1929, p. 227 ; VERVOORT, 1947, p. 153.

Female. Length, 2.47 mm ; cephalothorax, 1.70 mm, abdomen, 0.77 mm. The head separates from the 1st thoracic segment ; the last two thoracic segments are fused ;

the lateral corners of the last thoracic segment rounded; the lateral face of the thoracic segments are finely spinulose; the rostrum absent.

The abdominal segments and furca in the proportions 28 : 21 : 21 : 16 : 14 = 100; the genital segment about as long as wide; the ventral surface of the segment has irregular swellings on the genital area; the 2nd and 3rd gegments hirsute on the proximal margin on the ventral surface; the furcal rami about 1.3-times as long as wide.

The 1st antenna exceeds the end of the furca by distal four joints. The 2nd antenna with the exopodite 1.5 times as long as the endopodite. The mandible hirsute on the 2nd basal joint. The maxilla and maxillipede as those of *S. abyssalis*, but the 1st basal joint of the maxillipede has a row of long spines which is entirely absent in *S. abyssalis*, about the middle of the posterior margin.

The swimming legs have general resemblance to those of *S. abyssalis*.

Remarks. According to FARRAN the size of the animal varies from 1.85 to 2.0 mm in the West coast of the Ireland, and 2.13 to 2.45 mm in the Antarctic. WOLENDEN's *S. horridus* is identical with *S. spinosus*, which measured 2.35 mm in total length.

Occurrence. Ten females were taken in Sagami Bay, Nov. 1937, from the depth 1200–0 m.

Distribution. The species has been recorded from the West Coast of Ireland, Bay of Biscay, and Antarctic. The species has a fairly wide distribution in the deep waters of the oceans.

Spinocalanus magnus WOLFENDEN

(Fig. 15, *a–g*)

Spinocalanus magnus FARRAN, 1908, p. 27; WOLFENDEN, 1911, p. 216; G. O. SARS, 1925, p. 33; FARRAN, 1929, p. 227; SEWELL, 1929, p. 95; JESPERSEN, 1934, p. 52; VERVOORT, 1947, p. 150.

Female. Length, 2.50 mm; cephalothorax, 2.00 mm. The cephalothorax elongate ovate; the last thoracic segment not produced as that figured by G. O. SARS. The abdomen 4-segmented, the segments and furca in the proportions 36 : 14 : 11 : 18 : 21 = 100; the genital segment produced below; the genital part has irregular swellings; the furcal rami 1.5-times as long as wide.

The 1st antenna were broken off in all specimens. The 2nd antenna has the exopodite about as long as the endopodite; the other mouth parts as described by WOLFENDEN.

In the 1st leg the 2nd basal joint has several spinules on the inner distal margin. The 2nd leg has 3 jointed exopodite and 2-jointed endopodite, the 2nd and 3rd joints of the exopodite, and the 2nd joint of the endopodite are each furnished with a row of spinules. The 3rd and 4th legs have each 3-jointed exopodite and endopodite. The 5th leg absent.

Male. Length, 2.13 mm. The general appearance as in the female. The abdomen is contained 2.7-times in the length of the cephalothorax; the abdomen 5-segmented, the segments and furca in the proportions 15 : 19 : 14 : 21 : 10 : 21 = 100; the furcal rami 2-times as long as wide. The 1st antenna reaches back to the end of the furca. The 1st to 4th legs as those of the female. The 5th pair of legs have 3-jointed exopodite and 2-jointed endopodite on each side.

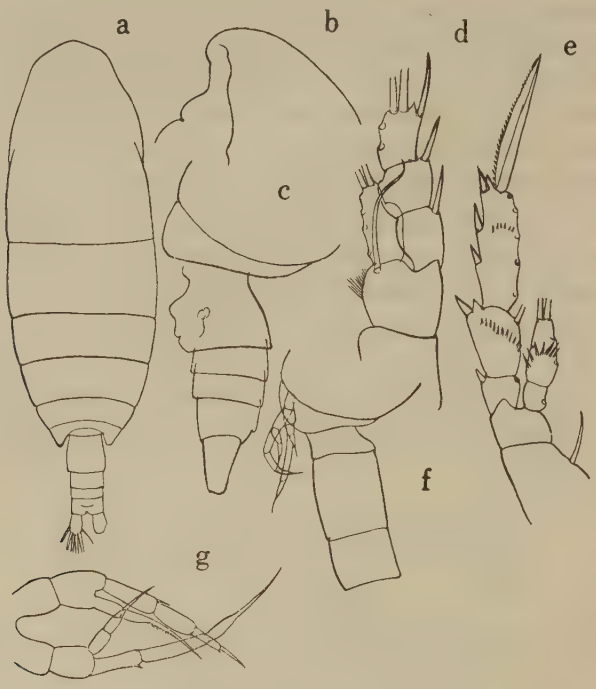

Fig. 15. *Spinocalanus magnus* WOLFENDEN.
a, Female: dorsal aspect; *b*, head. lateral aspet; *c*, last thoracic segment and abdomen, lateral aspect; *d*, 1st leg; *e*, 2nd leg; *f*, Male: last thoracic segment and abdominal segments, lateral aspect; *g*, 5th pair of legs.

Occurrence. Seven females and one male from the depth 1000 m to the surface in both Sagami and Suruga Bay.

Distribution. It has been recorded from the deep waters of the Pacific, Atlantic and Indian Oceans.

Spinocalanus angusticeps G. O. SARS

(Fig. 16, *a-f*)

Spinocalanus angusticeps SARS, 1920, p. 3; G. O. SARS, 1925.

Female. Length, 2.33 mm ; cephalothorax, 1.86 mm ; abdomen, 0.47 mm. The head separates from the 1st thiracic segment ; the last two thoracic segments separate. The head contracts laterally in front when viewed from the dorsal. The lateral corners of the last thoracic segment rounded. The rostrum absent.

The abdomen 4-jointed, contained 4-times in the length of the cephalothorax ; the segments and furca in the proportions 37 : 19 : 16 : 14 : 14 – 100 ; the genital segment produced rectangularly below ; the furcal rami slightly wider than long ; the 1st to 3rd segments are furnished with fine teeth on the distal margin.

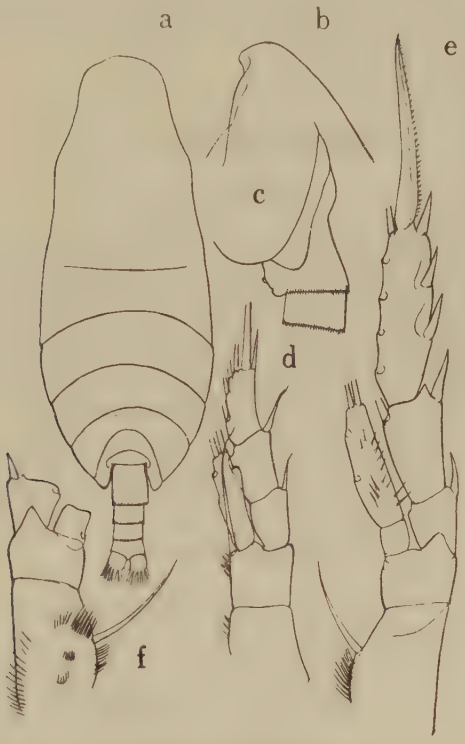

Fig. 16. *Spinocalanus angusticeps* G. O. SARS.
a, Female ; dorsal aspect ; *b*, head, lateral aspect ; *c*, last thoracic segment and gehital segment, lateral aspect ; *d*, 1st leg ; *e*, 2nd leg ; *f*, basal joints of 4th leg.

The 1st antenna broken off in the distal part ; the 2nd joint is very long, about 3-times as long as the 3rd. The 2nd antenna has the exopodite shorter than the endopodite (30 : 35). The mandible has the exopodite about as long as the endopodite. The 1st maxilla has a large exopodite, carrying nine setae ; the endopodite small ; the outer lobe has nine setae. The 2nd maxilla without any noticeable feature. The maxillipede has a long endopodite resembling that of *S. abyssalis*.

The 1st leg has 3-jointed exopodite and 1-jointed endopodite. The 2nd leg has 3-jointed exopodite and 2-jointed endopodite; the 2nd joint of the endopodite is furnished with spines on the posterior surface. The 3rd and 4th legs have each 3-jointed endopodite; the 2nd and 3rd joint of the endopodite are furnished with spinules on the posterior surface; the 1st basal joint is furnished with hairs as shown in the figure.

Remarks. The species is easily recognised by the peculiar shape of the head.

Occurrence. Three females in the vertical hauls from the depth 1000 m to the surface in Sagami, Nov., 1937.

Distribution. The species has been recorded from the temperate region of the Atlantic near the Azores Islands.

Spinocalanus longipes sp. nov.

(Fig. 17, *a–f*)

Male. Length, 1.59 mm; cephalothorax, 1.16 mm; abdomen, 0.43 mm; so the abdomen is contained 2.7-times in the length of the cephalothorax. The head separates from the 1st thoracic setment, and the 4th incompletely from the 5th. The last thoracic segment slightly emarginate on the postero-lateral corners. The rostral filament absent. The abdomen 5-segmented; the segments and furca in the proportions 10 : 31 : 22 : 21 : 6 : 10 = 100; the furcal rami slightly longer than wide (5 : 4).

The 1st antennae extend about to the distal margin of the abdominal segment; the right antenna 22-jointed, the joints 8–9–10, 20–21 are fused; the left antenna 23-jointed, the joints 20 and 21 are separate; joints of the antenna measured along the posterior margin are in the following proportions:

Joint	1	2	3	4	5	6	7	8–9–10	11	12	13	14	15	16	17
	71	54	24	30	30	30	30	95	29	36	35	35	42	48	47
	18	19	20	21	22	23	24	25							
	54	54	47	36	48	47	54	24 = 1000							

The 2nd antenna has the exopodite 1.25 times as long as the endopodite. The mandible has a robust and round 2nd basal joint; the exopodite about as long as the endopodite. The maxillae reduced. The maxillipede resembles that of *S. abyssalis*; the 2nd joint of the endopodite is long; the 5th joint comparatively large, and carries two long setae and two short ones.

The 1st leg with 3-jointed exopodite and 1-jointed endopodite. The 2nd to 4th legs broken off; the basal joints are furnished with spinules.

The left 5th leg is long and slender, it is much longer than the combined length of the abdominal segments and furca, the 2nd basal joint has a rudimentary endopodite; the right leg short, about as long as the 1st basal joint of the left leg.

Remarks. Although the swimming legs which is the important character of the genus were broken off, the absence of the rostrum, and the features of the joints of the maxilliped, may indicate that the specimen belongs clearly to the genus *Spinocalanus*. The 5th pair of legs are quite different from those hitherto been described and figured.

Occurrence. One male in the vertical haul from the depth 1000 m to the surface in Sagami, Nov., 1937.

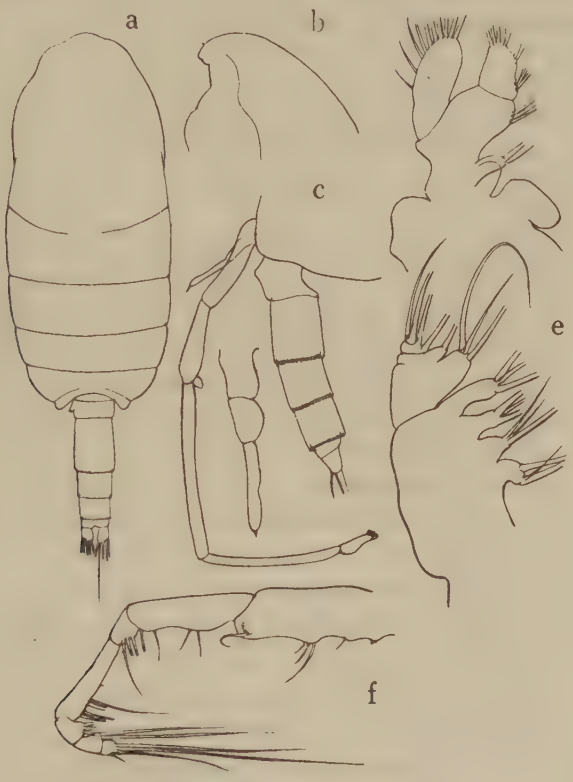

Fig. 17. *Spinocalanus longipes* sp. now.
a, Male: dorsal aspect; *b*, head, lateral aspect; *c*, last thoracic segment and abdomen, lateral aspect; d, 1st maxilla; *e*, 2nd maxilla; *f*, maxillipede.

Genus *Monacilla* G. O. Sars, 1905

G. O. Sars described in 1905 a new species of Calanoida under the name *Monacilla typica*. Farran (1908) described *Oxycalanus spinifer* which is congeneric with the former. In 1909 A. Scott described two species *Oxycalanus semispinus* and *Monacilla dubia*. But according to Sars these two species are two sexes of *Monacilla typica*. In the present collection two species were collected, namely *Monacilla typica* G.O. Sars

and *M. gracilis* WOLFENDEN. The genus has the following characters: the head fused with the 1st thoracic segment, and so are the 4th with the 5th; the rostrum consists of two strong spines, directing ventrally; the abdomen 4-jointed; the 1st antenna 23-jointed, the joints 8 and 9, 24 and 25 are fused; the 2nd antenna, maxilla and maxillipede are of *Pseudocalanus* type; the 1st to 4th legs as in *Spinocalanus*; the distal joint of the endopodite of the 2nd to 4th legs has 5, 6, 6 setae respecitively; in this the genus is closely related to *Spinocalanus* but, the spinulation on the faces of the joints are reduced than in *Spinocalanus*; the 5th leg absent in the female, in the male 5th legs 5-jointed on each side and uniramose.

Monacilla typica G. O. SARS

(Fig. 18, *a–h*)

Monacilla typica SARS, 1905, p. 9; *Oxycalanus spinifer* FARRAN, 1908, p. 35; *O. semispinus* A. SCOTT, 1909, p. 35; *Monacilla typica* G. O. SARS, 1925, p. 38; VERVOORT, 1947, p. 158.

Female. Lengh, 2.25 mm; cephalothorax, 1.77 mm, abdomen, 0.48 mm. The cephalothorax oblong ovate; the head separates from the 1st thoracic segment; the last two thoracic segments fused; last thoracic segment slightly asymmetrical; the lateral corners of the segment rounded, and slightly produced posteriorly; the rostrum produced into well defined sharp point.

The abdomen 4-jointed, the segment and furca in the proportional lengths 40 : 16 : 15 : 11 : 18 = 100; the genital segment aymmetrical: the right side is slightly swollen, there is a protuberance on the ventral surface of the right side; the furcal rami 1.7-time as long as wide, the outermost seta is short, this is not figured by SARS.

The 1st antenna 24-jointed, reaches back to the distal margin of the 3rd abdominal segment. The 2nd antenna, mandible and maxilla as figured by SARS. The maxillipede has a transverse row of spinules on the posterior margin about the middle of the 1st basal joint as illustrated by A. SCOTT.

The 1st leg has 3-jointed exopodite and 1-jointed endopodite; the outer margin of the 1st has basal joint has groups of stiff hairs. The 2nd leg has 3-jointed exopodite and 2-jointed endopodite; the 2nd joint of the endopodite has two rows of spinules; the 1st basal joint is furnished with spinules; the terminal spine of the exopodite finely serrated. The 3rd leg has 3-jointed exopodite and endopodite; the 2nd and 3rd joints of the exopodite and endopodite are furnished with rows of spinules. The 4th leg has 3-jointed exopodite and endopodite; the 2nd and 3rd joints of the exopodite and endopodite are furnished with groups of spinules as shown in the figure; the 1st basal joint has a transverse row of long acicular spines on the posterior surface about the middle of the joint only on the left side; the acicular spines not extend beyond the distal margin of the 1st basal joint; the terminal spine of the exopodite is coarsely serrated.

Remarks. The present specimen agrees well with the description and figures given by several authors except that the head and 1st thoracic segment are separate.

Occurrence. Twenty five females in Sagami and Suruga Bay, from the depth 1000 m to the surface.

Distribution. The species has a wide distribution in the Atlantic, Pacific and Indian Oceans.

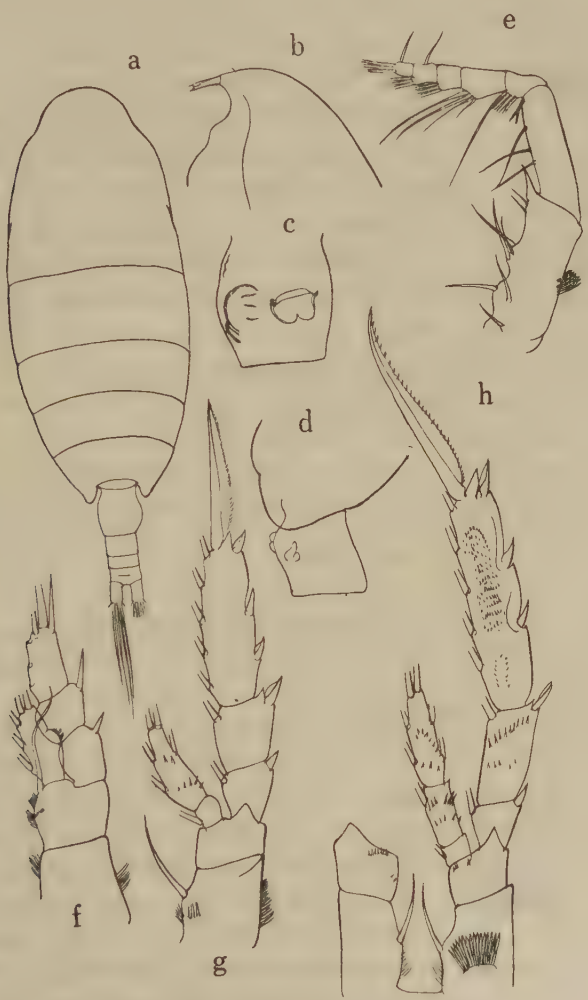

Fig. 18. *Monacilla typica* G. O. SARS.
a, Female: dorsal aspect; *b*, head, lateral aspect; *c*, genital, ventral aspect; *d*, last thoracic segment and genital segment, lateral aspect; *e*, maxillipede; *f*, 1st leg; *g*, 2nd leg; *h*, 4th legs.

Monacilla typica var. *asymmetrica*, var. nov.

(Fig. 19 *a*)

Fig. 19. *Monacilla typica* var. *asymmdtrica*, var. nov. *a*, Female: 4th legs.

Female. Length, 2.27 mm; cephalothorax, 1.77 mm; abdomen, 0.50 mm. The specimen agrees quite well with *M. typica* SARS except that there is a transverse row of short and slender acicular spines on the posterior surface of the 1st basal joint of the right 4th leg; the specimen may be referred to *M. gracilis* WOLFENDEN in having acicular spines on either side of the 1st basal joint of the 4th leg; but the shape of the genital segment differs from that of *M. gracilis*; the genital segment has a swelling on the right side as in *M. typica*, but the protuberance on the right side of the genital orfice is absent.

Occurrence. Two females from Sagami Bay in the vertical hauls from the depth 1000 m to the surface.

Monacilla gracilis (WOLFENDEN)

(Fig. 20, *a–c*)

Oxycalanus gracilis WOLFENDEN, 1911, p. 221.

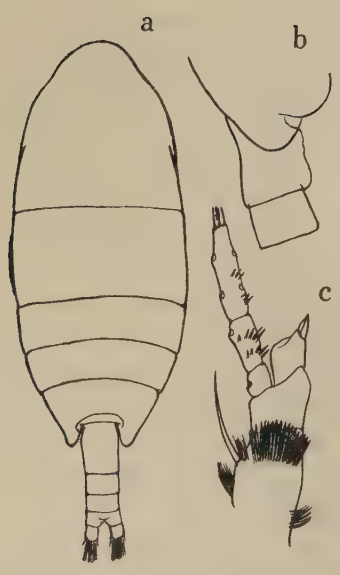

Fig. 20. *Monacilla gracilis* WOLFNDEN. *a*, Female: dorsal aspect; *b*, last thoracic segment and genital segment, lateral aspect; *c*, 4th leg.

Female. Length, 2.25 mm; cephalothorax, 1.75 mm; abdomen, 0.50 mm. The general appearance as in *M. typica*; the abdomen 4-segmented, the segments and furca in the proportional lengths 38 : 17 : 17 : 14 : 14 = 100; the genital segment symmetrical, not produced below as in *M. typica*; the furcal rami 1.3-times as long as wide.

The 1st antenna slightly exceeds the end of the furca when pressed against the body. The 2nd antenna has the exopodite as long as the endopodite. The mandible has the exopodite as long as the endopodite. The 1st maxilla has the following spinulation; the outer lobe, 9; the exopodite, 11; the endopodite, 6, 5, 4; the 2nd basal, 5; the 3rd inner lobe, 4; the 2nd inner lobe, 5; the 1st inner lobe, 14 setae. The 2nd maxilla and maxillipede have no interesting feature.

The 1st to 3rd swimming legs are as those of the foregoing species. The 4th pair of legs symmetrical; the transverse row of acicular spines exceeds

the distal margin of the 1st basal joint of the legs; the number of acicular spines about 18.

Remarks. The specimen, though larger in size, agrees fairly well with the WOLFENDEN's examples collected from the deep waters of the tropical Atlantic in Südpolar-Expedition. The species, though closely resembles *M. typica*, differs from it in the shape of the genital segment, and in having acicular spines on the 1st basal joint of the 4th pair of legs.

Occurrence. One adult female from Sagami in the vertical haul 1000 m to the surface, Nov., 1937.

Genus *Drepanopsis* WORFENDEN, 1911

The genus was created by WOLFENDEN. At present it comprises only one species, *Drepanopsis frigidus*, taken from the Antarctic region. The generic characters are as follows: head fused with the 1st thoracic segment; last two thoracic segments are separate; rostrum or rostral filments absent; 1st antenna 24-jointed, the joints are very setose; 2nd antenna with the endopodite longer than the exopodite; mandible with a small endopodite; 1st and 2nd maxillae and maxillipede are of *Paracalanus*-type; 1st to 4th legs as those of *Spinocalanus*, but the number of spines on the faces of the joints much reduced; 5th pair of legs 3-jointed, with two apical setae. The genus *Farrania* created by SARS is identical with *Drepanopsis* WOLFENDEN. The presence of four setae on the 3rd joint of the exopodite of the 2nd and 3rd legs indicate that the genus *Farrania* is not allied to the genus *Spinocalanus*, but to *Clausocalanus* or *Pseudocalanus*.

Drepanopsis frigidus WOLFENDEN

(Fig. 21, *a–k*)

Drepanopsis frigidus WOLFENDEN, 1911, p. 245; FARRAN, 1929, p. 232; SEWELL, 1929, p. 96; VERVOORT, 1951, p. 61, figs. 34–39.

Female. Length, 2.88–2.95 mm. The body oblong ovate in dorsal view. The head fused with the 1st thoracic segment, the 4th thoracic segment separates from the 5th; the lateral corners of the last thoracic segment produced triangularly, but the apex is not so sharp as figured by WOLFENDEN. The rostrum is represented by two small points when viewed from the ventral.

The abdomen 4-segmented and is contained about 6-times in the length of the cephalothorax; the segment and the furca in the proportions 42 : 8 : 8 : 13 : 29 = 100; the genital segment 1.4-times as wide as long, and produced considerably below; the first three segments are fringed with very minute teeth on the distal margin; the furcal rami 1.5-times as long as wide.

The 1st antenna 24-jointed, reaches back to the end of the 3rd abdominal segment;

the joints measured along the posterior margin are in the following proportions:

Joint	1	2	3	4	5	6	7	8–9	10	11	12	13	14	15	16	17
	31	55	27	31	31	31	34	55	31	34	34	48	52	55	52	51

	18	19	20	21	22	23	24	25
	51	51	41	34	48	52	45	27 = 1000;

Fig. 21. *Drepanopsis frigidus* WOLFENDEN.
a, Female: dorsal aspect; *b*, head, lateral aspect; *c*, abdomen, dorsal aspect; *d*, last thoracic segment and genital segment, lateral aspect; *e*, rostrum, ventral aspect; *f*, 2nd antenna; *g*, mandible blade; *h*, 1st maxillia; *i*, 1st leg; *j*, 2nd leg; *k*, 4th leg.

the joints are furnished with slender setae. The 2nd antenna has the exopodite about as long as the endopodite; the 2nd joint of the exopodite has two protuberances which carry each a short marginal seta. The mandible has a small endopodite; the biting part is furnished with groups of fine spinules. The 1st maxilla has the following spinulation: the outer lobe has 9 setae, the exopodite 11 setae, the endopodite 4, 4, 6 setae. The 2nd maxilla has a row of spinules on the 2nd and 3rd lobe; the setae

arising from the endopodite are unmodified. The maxillipede as shown in the figure; the basal joints are furnished with rows of spinules on the anterior margin.

The 1st leg has 3-jointed exopodite and 1-jointed endopodite; the 1st basal joint has two groups of spinules on the outer margin. The 2nd leg has 3-jointed exopodite and 2-jointed endopodite; the 1st and 2nd joints of the endopodite are furnished with spinules on the posterior surface; the terminal spine of the exopodite has 29 serrations. The 3rd and 4th leg have each 3-jointed exopodite and endopodite; the joints of the endopodite are furnished with groups of spinules on the posterior surface; the terminal spine of the exopodite has 28 and 43 serrations respectively. The 5th pair of legs 3-jointed; the distal joint has two spines; the inner one is about as long as the combined lengths of the three joints taken together.

Remarks. The specimen agrees well with the description given by WOLFENDEN. FARRAN's specimen from the Antarctic has the total length 2.58–2.88 mm; SEWELL's specimen from the Indian Seas measured 2.28 mm, and has a backwardly pointing median spine instead of bifid rostrum. VERVOORT's specimen from the Antarctic measured 2.90 mm, and has a rostral median spine. The present specimen agrees quite well with those described except the stupid rostrum and longer abdomen and 1st antenna.

Drepanopsis orbus sp. nov.

(Fig. 22, *a–k*)

Female. Length, 3.56 mm; cephalothorax, 2.79 mm; abdomen, 0.77 mm; the cephalothorax elongate ovate; the head separates from the 1st thoracic segment; the 4th and 5th thoracic segments are incompletely separate; the lateral corners of the last thoracic segments are produced into sharp points; the rostrum absent.

The abdomen 4-jointed; the segments and furca in the proportions: 38 : 18 : 16 : 15 : 13 – 100; the genital segment as long as wide; the furcal rami as long as wide; the outer-marginal seta of the furca slender and short; the first three abdominal segments are furnished with fine spinules on the distal margin.

The 1st antenna 24-jointed, extends to the distal margin of the genital segment, the joints are in the following proportional lengths:

Joint	1	2	3	4	5	6	7	8–9	10	11	12	13	14	15	16	17
	58	81	38	35	38	35	36	55	32	32	35	46	49	49	49	49

	18	19	20	21	22	23	24	25
	46	46	38	38	32	35	32	17 = 1000;

the setae on the joints are slender as in *D. frigidus*. The 2nd antenna has the exopodite about as long as the endopodite (42 : 44); the 2nd joint of the exopodite has three prominences on the anterior margin, and they carry each a seta; the 1st basal has a row of hairs. The mandible as those of *D. frigidus*. The 1st maxilla has the

following spinulation on the various parts: the outer lobe has 7 long and 2 short setae; the exopodite is very small, carries 11 setae; the endopodite has 7, 5, 4 setae; the 2nd basal joint has 5 setae; the 3rd and 2nd inner lobe have each 4 setae; the 1st inner lobe has 14 setae. The 2nd maxilla as that of *D. frigidus*. The maxillipede robust, and in the main features resembles that of *D. frigidus*.

Fig. 22. *Drepanopsis orbus* sp. nov.
a, Female: dorsal aspect; *b*, head, lateral aspect; *c*, last thoracic segment and genital segment, lateral aspect; *d*, anal segment and furca, dorsal aspect; *e*, maxillipede; *f*, 1st leg; *g*, 2nd leg; *h*, endopodite of 4th leg; *i*, Male: last thoracic segment and genital segment; *j*, 5th pair of legs; *k*, terminal joint of right 5th leg.

The 1st leg has 3-jointed exopodite and 1-jointed endopodite; the joints of the endopodite slender; the outer-edge spines of the exopodite long, there is a small protuberance on the outer margin about one-third of the terminal joint of the exopodite; the 1st basal joint protuberates on the outer distal corner. The 2nd leg has

3-jointed exopodite and 2-jointed endopodite; the 1st basal joint is furnished with stiff hairs on the outer and also in the inner margin; the 2nd basal joint has a row of spines near the junction with the exopodite; the endopodite has a group of spines on the 1st joint, and three groups of spines on the 2nd joint; the terminal spine of the exopodite has 39 serrations. The 3rd leg has 3-jointed exopodite and endopodite, and has similar features as those of the 2nd leg; the terminal spine of the exopodite has 34 serrations. The 4th leg has 3 jointed exopodite and endopodite; there is no stiff hair on the outer margin of the 1st basal joint, and no row of spine on the 2nd basal joint; the endopodite is furnished with very minute spinules on the posterior surface; the terminal spine has 42 serrations. The 5th leg entirely absent.

Male. Length, 3.01 mm; cephalothorax, 2.30 mm, abdomen, 0.71 mm; the general appearance as in the female. The abdomen 5-segmented, the segments and fruca in the proportional lengths: 13 : 26 : 20 : 23 : 3 : 15—100: the furcal rami about 1.3-times as long as wide.

The 1st antenna 21-jointed, reaches back to the middle of the 2nd abdominal segment; the joints are in the following proportions:

Joint	1	2	3	4	5	6	7	8-9-10	11	12-13	14	15	16	17
	31	73	35	31	34	34	34	57	31	31	34	42	42	50	50	50

18	12	20	21	22	23	24-25		
50	54	46	56	42	50	38	15 = 1000;	

the joints 8-9-10, 12-13 and 24-25 are fused; the joints 20 and 21 are fused on the right side; the 3rd joint has a very long distal seta. The 2nd antenna as in the female, and bears three prominences on the 2nd joint of the exopodite. The mandible has a robust 2nd basal joint; the exopodite about as long as the endopodite (13 : 15). The 1st maxilla has a large endopodite bearing 10 setae; the endopodite reduced, has 11 setae; the 1st inner lobe much reduced. The 2nd maxilla reduced; the first three lobes are furnished with only poor setae. The maxillipede as in the female.

The 1st to 4th swimming legs as those of the female. The 5th pair of legs slender, consist of 2-jointed basal joint and 3-jointed exopodite and 1-jointed endopodite; the right leg is longer than the left, the distal joint of the right leg is furnished with hairs as shown in the figure.

Remarks. The specimen differs from *D. frigidus* in the segmentation of the cephalothorax, and in the absence of the 5th leg in the female. But in other respects the specimen is so closely allied to the genus *Drepanopsis* that I dare regard the present specimen as congenerous with *Drepanopsis*. The structure of the male 5th legs also indicates a close affinity to *Drepanopsis*.

Occurrence. Three females and one male in the vertical hauls from 1000 m to the surface in Sagami Bay.

Genus *Tanyrhinus* FARRAN, 1936

FARRAN created a new genus *Tanyrhinus* to accomodate a small Calanoida closely resembling to *Mimocalanus* FARRAN. The generic characters are as follows: the head and the first thoracic segment are separate; the 4th and the 5th thoracic segments are separate; the abdomen 4 jointed; the 1st antenna 23-jointed, the joints 8–9 and 24–25 being fused; the 2nd antenna as in *Mimocalanus*, with two setae on the inner margin of the 2nd joint of the exopodite; the mandible with a large two-branched palp; the endopodite longer than the exopodite; the maxilla as in *Mimocalanus* and *Spinocalanus*; the 2nd maxilla as in *Paracalanus*, except that the 4th lobe is longer than the 1st; the maxillipede is of *Mimocalanus* type, with well developed setae; the 1st and 2nd joints of the exopodite of the 1st leg without inner marginal seta, and the terminal joint of the endopodite of the 2nd to 4th legs have four inner marginal setae; the 5th leg absent.

The genus comprises only one species, *Tanyrhinus naso* FARRAN. Up to the present time no example of the genus has not been met with in the Izu region.

Ryocalanus TANAKA, 1956

Cephalothorax elongate; the head is separate from the 1st thoracic segment, and so are the 4th from the 5th; rostrum stout, one-jointed; the abdomen 5-jointed in the male; 1st antenna 24-jointed, the joints 8 and 9 are fused; in the male right antenna is transformed into a grasping organ, the joints 23 and 24 are fused, the hinge exists between the joints 22 and 23. 2nd antenna as in *Drepanopsis*, with three setae on the inner margin of the 2nd joint of the exopodite, the 1st basal joint has a brush of hairs in posterior aspect. Mandible with a slender palp, the endopodite 2-jointed, shorter than the exopodite. 1st maxilla as in *Mimocalanus*, but in the exopodite, 2nd basal joint and lobes are slender. 2nd maxilla as in *Pseudocalanus*, the 5th lobe is the largest. Maxillipede as in *Drepanopsis*, except that the distal two joints are furnished with well developed setae on the outer margin. 1st to 5th swimming legs as in *Spinocalanus* and *Monacilla*, except that the distal joint of the exopodite of the 1st leg has a spine on the outer margin about the middle of the joint. The 5th legs of the male as in *Pseudocalanus*.

Ryocalanns infelix TANAKA

Ryocalanus infelix TANAKA, 1956, p. 3, figs. 1 and 2.

Male. Length, 2.18 mm; cephalothorax, 1.93 mm, abdomen, 0.25 mm; the head separates from the 1st thoracic segment; the last two thoracic segments are separate; the cephalothorax elongate ovate, the head contracts anteriorly; the last thoracic segment produced into an acute spine on each side, directing ventrally; the ventral

margin of the segment is furnished with fine spinules; the rostrum one-pointed, and acute, there is, in lateral view, a notch on the posterior surface near the distal end.

The abdomen 5-jointed, the segments and the furca in the proportional lengths 32 : 18 : 11 : 7 : 14 : 18 100; the 1st abdominal segment about half as long as wide, and is furnised with fine spinules on the distal corner of the right side, the 2nd to 4th segments are fringed with spinules on the distal margin; the furcal rami wider than long, carries five setae, of which the outermost is short and slender, the dorsal surface of the rami furnished with minute spinules, the appendicular seta short.

The left 1st antenna broken off in the distal part, the joints 8–9 are incompletely fused on the anterior margin, the joints 1 to 15 are in the proportions:

Joint	1	2	3	4	5	6	7	8–9	10	11	12	13	14	15
	20	14	5	5	5	5	4	7	5	6	6	6	6	6

The right antenna forming a grasping organ, extends to the distal margin of the 2nd thoracic segment; the joints 8–9 are entirely fused, the joints 18 to 22 are inflated; each joint with a distal seta and an aesthetask on the anterior margin; joint 20 has a seta on the posterior margin, the joint 21 has fine denticles and a seta on the posterior margin; the joint 22 is highly chitinised on the posterior margin and has a short distal seta, the joint 23 and 24 are incompletely separate, and forms a comb-like projection furnished with 19 denticles on the posterior margin; there is a minute seta at the junction with the 24th joint; the 24th joint has also a distal seta on the anterior and posterior margins; the joint 25 has five setae and an aesthetask; the joints 12 to 19 are furnished with hairs on the posterior margin.

The 2nd antenna has the exopodite about as long as the endopodite; the exopodite 7-jointed, the joint 2 has three marginal setae; the distal joint of the endopodite has 8–6 setae. The mandible has the exopodite slightly longer than the endopodite; the exopdite 5-jointed; the endopodite 2-jointed, and has ten setas on the 2nd joint; the 2nd basal joint carries three setae; the cutting edge has eighe teeth. The 1st maxilla well developed; the outer lobe has four long and two short setae; the exopodite has 7 + 4 + 4, the 2nd basal five; the 3rd inner lobe four; the 2nd inner lobe five; the 1st inner lobe fourteen spines. The 2nd maxilla is of normal type; the 1st lobe has four setae, the 2nd to 5th have each three setae; the 6th lobe has two setae; of which the distal one is short; the endopodite has six setae; the 1st basal has an outer marginal seta. The maxillipede slender; the outer marginal seta on the 4th and the 5th joint of the endopodite long; the 2nd joint of the endopodite is the largest.

The 1st leg has 3-jointed exopodite and 1 jointed endopodite; the outer edge spine of the joints of the exopodite are long; the 3rd joint has four inner marginal setae, and two outer marginal spines; the distal half of the outer margin is hollowed; the endopodite has a process on the proximal outer margin; the 2nd basal joint has a row of spinules on the inner distal margin at the base of the endopodite; the exopodite of the right leg has an abnormal structure: the inner marginal setae on the

2nd and 3rd joint of the exopodite are converted into strong spines. The 2nd leg has 3-jointed exopodite and 2-jointed endopodite; the joints of the exopodite, especially the 2nd and the 3rd joints are densely covered with fine spinules on the posterior surface; the 3rd joint has five inner marginal setae; the posterior surface of the joints of endopodite are furnished with groups of spinules; the terminal spine of the exopodite has 23 teeth which are connected with a sort of thin lamella; the outer margin of the 2nd joint and the proximal section of the 3rd joint of the exopodite have, beside acicular spines, a row of slender and curved spines as shown in the figure; this arrangement of the spinules is observed also in the exopodite of the 3rd and 4th legs. The 3rd leg has 3-jointed exopodite and endopodite; the joints of the exopodite and endopodite are furnished with spinules as in the 2nd leg; the terminal spine of the exopodite has 25 teeth; the 3rd joint of the endopodite has six setae in all. The 4th leg has 3-jointed exopodite and endopodite; the structure as in the 3rd leg; the terminal spine of the exopodite is very finely serrated.

The 5th legs consist of five joints; the left leg much longer than the right; the terminal joint has two apical spines; the right leg has an outer edge spine on the 3rd joint; the terminal joint has two unequal apical spines.

Remarks: The specimen appears to be allied to *Autanepsis*, described by WOLFENDEN in the report of the "Süd-polar Expedition" in the structure of the swimming legs, but mouth parts are entirely different. The grasping antenna of the present specimen differs from those found in the tribe Heterarthrandria which has a movable articulation between the joints 18 and 19. The structure of the 1st to 4th legs resemble those of Pseudocalanidae, yet the peculiar structure in the grasping antenna has not, so far as I am aware, been reported, and I accommodate, at the present time, this curious specimen in the new genus under the name *Ryocalanus infelix* in memory of my only son who lost his life early in his boyhood. The description and figures of the species were published in "Breviora," Museum of Comparative Zoology of Harvard University, No. 64, October 11, 1956.

Occurrence. One adult male in the vertical haul from the depth 1410 m to the surface, in Suruga Bay, Nov., 1938.

With the Author's Compliments

Further Note on *Calanus tonsus* BRADY in Japanese Waters

O. TANAKA

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Further Note on *Calanus tonsus* BRADY in Japanese Waters*

Otohiko TANAKA**

Abstract: In my previous paper (1954) I have noticed that *Calanus plumchrus* MARUKAWA is identical with *Calanus tonsus* BRADY. However, upon examination of the specimen of *C. tonsus* taken from the Antarctic *C. plumchrus* was proved to be a characteristic copepod of the North Pacific, and is distinct from *C. tonsus* BRADY of the Antarctic.

1. Introductions

I have reported (In 1954) that *C. plumchrus* MARUKAWA is identical with *C. tonsus* BRADY. Sometime after that NAKAI of the Tokai Regional Fisheries Laboratory informed me by his personal communication that *C. tonsus* of the Antarctic is distinct from *C. tonsus* of the North Pacific. The Antarctic specimen has been reported by WOLFENDEN (1908) and FARRAN (1929); the North Pacific specimen by CAMPBELL (1930. 34), BRODSKY (1938), DAVIS, (1949) and TANAKA (1953, 54, 56). MARSHALL (1955) recognized *C. plumchrus* MARUKAWA as a variety of *C. tonsus* under the name *C. tonsus var plumchrus*. Yet there remain undecided whether these two examples are identical.

Recently I had an opportunity to get the specimen of *C. tonsus* BRADY taken by the British-Australian-New Zealand Antarctic Expedition in the Antarctic waters. On comparing the Antarctic specimen with that of the North Pacific it revealed that these two examples are not identical, viz. the specimen from the Antarctic is identical with that originally described by BRADY, and that from the North Pacific is synonym of *C. plumchrus* described by MARUKAWA.

I am deeply indebted to Dr. W. VERVOORT who sent me the precious specimen of copepoda, and also to Mr. J. NAKAI who informed me on the validity of *C. plumchrus*.

2. Description of *Caranus tonsus* BRADY

The adult female from 48°23.5′S 146°29.5′E measured 4.07 to 4.24 mm, those from 44°05′S 147°35′E measured 3.66 to 3.75 mm. The immature female from the latter station measured 3.27 and 3.47 mm.

Adult female. Total length 4.07 mm. Cephalothorax and abdomen are in the proportional lengths 128 : 35. Body rather robust (Fig. 1. A). Head separates from thoracic segment. Frontal margin of head broadly rounded, (Fig. 1. B) but slightly produced when viewed in dorsal aspect. 4th and 5th thoracic segments are distinctly separated. Posterio-lateral border of 5th thoracic segment broadly rounded in lateral aspect; (Fig. 1, C) in dorsal view the pos-

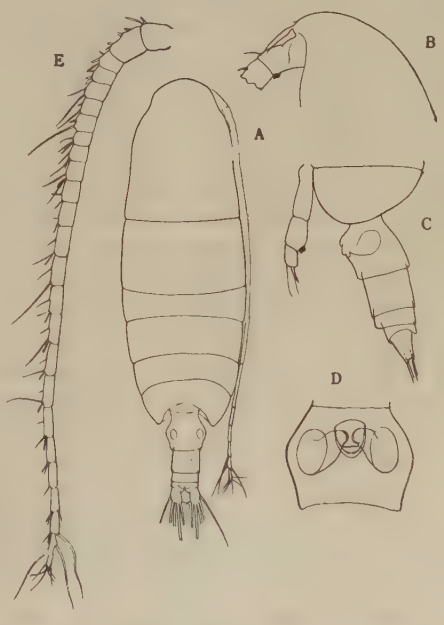

Fig. 1. *Calanus tonsus* BRADY. Female: A, dorsal view, ×19; B, head, lateral view, ×33; C, 5th thoracic segment and abdomen, lateral view, ×33; D, genital segment, ventral view, ×67; E, right 1st antenna, ×27.

* Received March 10, 1956.

** The Fisheries Laboratory, Kyushu University

terior corners of the 5th thoracic segment are bluntly produced. Rostrum is composed of two slender filaments.

Abdomen 4-segmented. The segments and furca are in the proportinal lengths 40 : 26 : 11 : 3 : 20 = 100. Genital segment laterally produced, about as long as wide in dorsal aspect (14 : 15) (Fig. 1, D). Genital opening produced slightly below; genital flap rather small. Furcal rami 1.5 times as longas wide.

1st antenna 25-jointed, entends about to the distal end of the anal segment (Fig. 1, E). The separation between 8th and 9th joints is distinct. The joints have the following proportional lengths:

1	2	3	4	5	6	7	8	9	10
50	68	35	28	30	30	32	25	25	37
11	12	13	14	15	16	17	18	19	20
38	43	50	50	50	50	52	48	50	42
21	22	23	24	25					
42	38	35	30	22 = 1,000					

Setae on the various joints are feeble.

2nd antenna has the endopodite slightly longer than the exopodite (47 : 44); inner marginal setae on the 1st and 2nd exopodal joints not feeble. 1st endopodal joint about 5 times as long as wide. 2nd exopodal joint has 6+1 setae on the external lobe (Fig. 2, A).

Mandible palp has the 2nd basal joint furnished with 4 setae on the inner margin. Exopodite 5-jointed, about as long as the 2-jointed endopodite. 1st endopodal joint with sack-shapec tubercle carrying 4 setae. Cutting edge has 6 groups of acute teeth and a plumose seta (Fig. 3, A, B).

1st maxilla has a rather small 1st inner lobe furnished with 10 strong and 4 slender spines. 2nd and 3rd inner lobe have each 4 setae. 1st outer lobe has 9 setae. Seta on the 2nd outer lobe was not observed. Exopodite with 11 setae. 2nd basal joint with 4 setae. 1st endopodal joint with 4, 2nd with 3rc with 7 setae (Fig. 2, B).

2nd maxilla has a plumose seta on the outer margin of the 1st basal joint. The number of setae on the various lobes and endopodal joints are as usual (Fig. 2, C).

Maxillipede has the 1st basal joint about as long as the 2nd basal joints. Exopodite

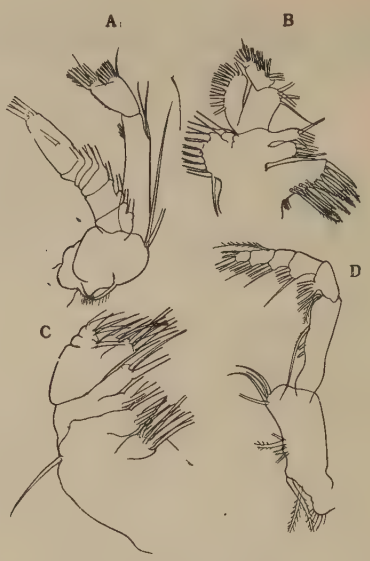

Fig. 2. *Calanns tonsus* BBADY. Female: A, 2nd antenna, ×67; B, 1st maxilla, ×67; C, 2nd maxilla, ×134; D, maxillipede, ×67.

about as long as the 2nd basal. The spinulation on the various lobes and joints are as usual (Fig. 2, D).

Swimming legs have each 3-jointed exopodite and endopodite. 1st and 2nd basal joints of 1st leg have each groups of short hairs on the anterior surface which can clearly be seen in lateral aspect (Fig. 3, C). 2nd to 4th legs have each 3 or 4 short pointed spines along the inner distal corner of the 2nd basal joint on the posterior surface (Fig. 3, D, E). Proportional lengths of the outer margin of the 3rd exopodal joint of 2nd to 5th legs devided by the outer marginal spine on the same joint as follows:

	2nd leg	3rd leg	4th leg	5th leg
prox. part	1.72	2.29	3.55	2.44
dist. part	1.00	1.00	1.00	1.00

5th leg has no inner marginal seta on the 1st basal joint, and inner margin of the joint smooth. Inner distal corner of 2nd basal joint had 3 spines on the right leg, and 4 spines on the left leg. 3rd endopodal joint has 2 outer marginal, 2 apical, and 2 inner marginal setae (Fig. 4, A). The short spines on the inner distal corner of the 2nd basal joint of 5th leg appear to be variable in number; they varied from 2 to 4 in the speci-

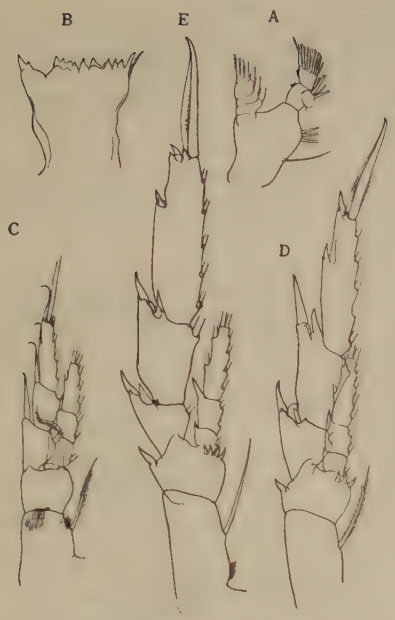

Fig. 3. *Calanus tonsus* BRADY. Female: A, mandible palp, ×67; B, cutting edge, ×134; C, right 1st leg, posterior surface, ×67; D, right 2nd leg, ×67; E, right 3rd leg, ×67.

mens observed; Wolfenden's specimen had 5 spines on the 2nd basal joint of right 5th leg.

Adult male unknown.

Immature female specimen in the 5th copepodid stage measured 3.27 and 3.47 mm. in total length. Abdomen 4-jegmentad. 5th pair of legs has 2-jointed exopodite and endopodite. 2nd to 5th legs are furnished with short pointed spines on the inner distal corner of the 2nd basal joint as in the adult female specimen.

The present specimen of *C. tonsus* from the Subantarctic is so closely allied to the specimen from the North Pacific that it is unable to distinguish them under the incomplete diagnosis given by BRADY. WOLFENDEN (1908) states in the report on copepoda of the National Antarctic Expedition that the only Calanus with which this shows agreement is, probably, BRADY's *C. tonsus*. But BRADY's description is so fragmentary that it may well be another species. The present specimen of the Antarctic agrees quite well with the description given by WOLFENDEN. FARRAN's specimen from the stations off New Zealand between lat. 30 to 40S and 50 to 60°S may perhaps be identical with WOLFENDEN's specimen from the Antarctic. The most distinguished characters which separates *C. tonsus* BRADY of the Antarctic from that of the North Pacific are: 1) inflated genital segment; 2) posession of acute teeth on the cutting edge of mandible; 3) posession of spines on the 1st inner lobe of 1st maxilla; 4) posession of a row of short spines on the inner distal corner of the 2nd basal joint of 2nd to 5th legs; 5) proportional lengths of the outer margin of the 3rd exopodal joint devided by the outer marginal spine of the same joint in 2nd to 4th legs. In the specimen of the North Pacific the segment is longer than wide (25:22); inner marginal setae on the 1st and 2nd exopodal joints of 2nd antenna are feeble (Fig. 4, B); cutting edge of mandible is smooth in the adult stage both in male and female (Fig. 4, C); 1st maxilla has no spine on the 1st inner lobe; setae on the various lobes of the 2nd maxilla are more feeble (Fig. 4, D); 2nd to 5th legs without any trace of spine on the inner distal corner of 2nd basal joint; proportional lengths of the outer margin of 3rd exopodal joint devided by the outer maaginal spine in 2nd to 5th legs as follows:

	2nd leg	3rd leg	4th leg	5th leg
prox.	1.00	1.04	1.20	1.36
dist.	1.00	1.00	1.00	1.00

Fig. 4. *Calanus tonsus* BRADY. Female: A, right 5th leg, posterior surface, ×67; *Calanus plumchrus* MARUKAWA Fmeale: B, 2nd antenna, ×67; C, mandible, ×67; 2nd maxilla, ×67.

Thus *C. tonsus* of the Antarctic is distintcly separated from that of the North Pacific recorded by McMURRICH (917), WILLEY (1920, WAILES (1929), JOHNSON (1932), CAMPBELL (1930, 1934), DAVIS (1949). MARUKAWA (1921) described separately *C. plumchrus*, a species of Calanus, from northern cold waters of Japan, which agrees quite well with *C. tonsus* reported by the American authors. However, *C. tonsus* from the North Pacific is in reality not synonym of *C. tonsus*, BRADY it must be replaced by the name *C. plumchrus* MARUKAWA.

VERVOORT (1951) suggests that BRADY confused *C. tonsus* and *C. actus* which is one of the common species of copepod in the Antarctic. But *C. tonsus* differs from *C. actus* in the shape of head and also in the swimming legs which have ho short spine on the inner distal cornor of the 2nd basal.

3. Geographical Distribution

C. tonsus appears to be distributed chiefly in the Subantarctic and has been recorded by BRADY (1883), WOLFENDEN (1908), T. SCOTT (1893) and FARRAN (1929). VERVOORT (1951) failed to detect it in the Antarctic. The species has been recorded also from the North Atlantic: THOMPSON (1890) at Madeira and Canary islands; THOMPSON and SCOTT (1898) from the Mid-Atlantic, 55°24′N; BIGELOW (1926) at the Gulf of Maine; WILSON (1932) at the Woods Hole region. BRADY (1833) recordee it from the North Pacific between Yokohama and Sandwitch islands. But these records of occurrence in the North Atlantic and North Pacific cannot be accepted without further evidence, as descriptions given by some of the old authors are very insuficient.

C. plumchrus is widely distributed in the cold waters of the North Prcific; the adult specimens both male and female have been recorded from deep layers in the adjacent waters of Japan.

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